ADDENDUM to the PROGRAM – RNA 2017
May 30 – June 3, 2017
Prague, Czech Republic

Corrections, withdrawals and new abstracts received after the program book was printed.

SESSION CHANGES

Friday, 2 June   09:00 – 12:30
Plenary Session 3: Splicing   Congress Hall

Withdrawn
62   Structural insight into the mechanism of splicing inhibition by modulators
          Vladimir Pena, Constantin Cretu
          Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Saturday, 3 June   14:30 – 17:15
Concurrent Session 11: RNA Catalysis/Folding   Meeting Hall 1 (floor 1)

Change of Presenting Author
133   Folding and splicing of group II intron ribozymes at the single molecule level
          Susann Zelger-Paulus, Roland K. O. Sigel
          Department of Chemistry, University of Zurich, Zurich, Switzerland

POSTER CHANGES

Withdrawn
211   Epigenetic switch of a macrosatellite repeat in colorectal cancer

Withdrawn
216   Long non coding RNAs as New Paradigm for Lung Cancer Pathogenesis

Withdrawn
220   Structural analyses of human and mouse NEAT1 lncRNAs suggest long-range RNA interactions contribute to paraspeckle architecture

Withdrawn
293   Cytosine methylation by DNMT2 facilitates stability and survival of HIV RNA in the host cell during infection

Withdrawn
305   RIsearch2-CRISPR: Predicting off-targets in CRISPR technology with energy models

Withdrawn
357   A strategy for selectively altering genetic information at the level of RNA

ADDED POSTERS

Added in topic area <u>Emerging and High-throughput Techniques</u> (in the space vacated by withdrawn 167)

167x   Nanopores allow direct sequencing of full-length RNA strands and modified RNA nucleotides.
<u>Elizabeth A. Snell</u>, *Daniel R. Garalde, Daniel G. Jachimowicz, Botond Sipos, Joseph H. Lloyd, Christopher Wright, Javier Blasco-Herrera, Anne-Marie Wadsworth, Clare Winter, Jemma Keenan, Anthony Warland, Jonah Ciccone, Nadia Pantic, David Alves, Andrew J. Heron, Lakmal Jayasinghe, Forrest Brennen, Jayne Wallace, James Clarke and Daniel J. Turner*
Oxford Nanopore Technologies Ltd., 4 Robert Robinson Avenue, Oxford, OX4 4GP, United Kingdom

  Nanopores are the only sequencing technology which can sequence a strand of native RNA directly without the need to convert to DNA. The direct RNA library prep developed by Oxford Nanopore Technologies Ltd is simple, whereby the RNA-specific motor-protein is ligated directly to the RNA molecule and then the prep is loaded on the flow cell and the RNA strand translocates through the nanopore, blocking the current at specific levels allowing the individual bases to be read and identified. Without the need to convert to DNA, long RNA molecules are able to be sequenced; for example we sequenced the full Human Rhinovirus (HRV) genome (~7 kb) as one full-length transcript using a single-stranded RNA protocol. Furthermore, the lack of amplification should reduce quantitation bias. To test this, and to ensure the ability to determine spliceosomal isoforms is straightforward, we sequenced Lexogen's SIRV panel and were able to discern different isoforms, but we did obverse some mismapping due to sequences with high degrees of similarity. However we experienced a high correlation of expected *versus* observed identified read counts when we sequenced the ERCC panel of 92 different transcripts (Spearman r = 0.97; p = 5.9e-56). We expect these both to improve with further enhancements to accuracy. The ability to sequence long read lengths allowed us to distinguish two highly abundant isozymes of GAPDH among *Saccharomyces cerevisiae* S228C direct mRNA transcriptome sequences. Perhaps most importantly, directly sequencing native RNA allows us to determine modified bases present based on distinct current-blocking signals. As such, we are able to discern both m6A and m5C bases from their canonical rAMP and rCMP counterparts in modified and unmodified f*Luc* transcripts which would normally be lost or converted by traditional sequencing methods. With Oxford Nanopore's Direct RNA sequencing kit, complete RNA strands can be sequenced on the MinION™, GridION™ and PromethION™ using a simple library prep, without the need to convert to double-stranded DNA.

214x   The Xist A-repeat fragment associates with chromatin through its conserved region independent of chromosomal accumulation.

Yuta Chigi[1], Hiroyuki Sasaki[2] and Takashi Sado[1]

[1]Department of Advanced Bioscience, Graduate School of Agriculture, Kindai University, Nara, Japan; [2]Division of Epigenomics and Development, Medical Institute of Bioregulation, Kyushu-University, Fukuoka, Japan.

X inactive-specific transcript (Xist) is a long non-coding RNA that plays an essential role for X chromosome inactivation. Although Xist RNA like common protein coding mRNAs is transcribed by RNA polymerase II, spliced and polyadenylated, it is retained in the nucleus and associates with the X chromosome it originates from. A previous study showed that hnRNP U is required for the accumulation of Xist RNA on the inactive X chromosome (Xi). However, we found that Xist RNA dissociated from Xi upon knockdown of hnRNP U was still localized in the nucleus with apparent association with chromatin. This raises the possibility that a distinct mechanism might operate to promote chromatin association of Xist RNA, which is independent of the hnRNPU-mediated mechanism. Analysis of the previous CLIP-seq data set in human cultured cells revealed that hnRNP U was poorly localized in the first 950-nt region of XIST RNA. Assuming that the corresponding region in mouse Xist RNA was also free of hnRNP U, we overexpressed RNA coding for this mouse sequence and examined its behavior. The result demonstrated that this fragment associated with chromatin through its conserved repeat sequence that is important for Xist RNA-mediated silencing. Our results suggest that the A-repeat captures and relocates local chromatin nearby sites, at which Xist RNA is initially loaded upon the initiation of X inactivation, into the core of heterochromatin domain.

220x   Structure-functional studies on human long non-coding RNA MEG3

Tina Uroda[1], Jean-Marie Teulon[2], Jean-Luc Pellequer[2], Marco Marcia[1]

[1] EMBL Grenoble, Grenoble, France; [2] Institut de Biologie Structurale, Grenoble, France

Human maternally expressed gene 3 (MEG3) is an imprinted, alternatively-spliced long non-coding RNA (lncRNA) possessing a key role as a tumor suppressor. MEG3 activates p53 inhibiting cell proliferation and it interacts with *Polycomb* group proteins controlling cell differentiation. Evidence suggests that MEG3 is highly structured and that its structure regulates its cellular functions. For instance, MEG3 is well conserved in mammals and has an unusually high GC content. Moreover, MEG3 forms putative secondary structure motifs, whose disruption impairs p53 activation (Zhang et al., 2010). Such motifs may also affect splicing efficiency and thus the abundance of its 27 splicing variants, which exhibit different p53 activation capacity (Zhang et al., 2010). Thus, there may be a correlation between exonic organization, structural architecture and function. Finally, MEG3 structure may participate in protein recognition, particularly *Polycomb* group proteins, which curiously do not possess canonical RNA binding domains.

While the physiological and medical relevance of MEG3 is known, its molecular mechanism still remains largely unexplained, partly because little is known of its biochemical and structural properties. Therefore, in our project we set out to determine MEG3 secondary and tertiary structure with an unconventional biochemical and biophysical approach.

We purified highly homogeneous MEG3 samples in non-denaturing conditions (Chillon et al., 2015), which allowed us to obtain the first experimentally-determined MEG3 secondary structure map by chemical probing (SHAPE). Such secondary structure map reveals that MEG3 is organized in highly-structured domains, which we are going to visualize in 3D by small-angle X-ray scattering and atomic force microscopy. By *in vivo* and *in vitro* functional assays we will now also establish precise correlations between these structural domains and the functional roles of MEG3 in chromatin remodelling and cell cycle regulation.

References:
Zhang X, et al. Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid: isoform structure, expression, and functions. Endocrinology 2010; 151: 939-47.
Chillon I, et al. Native Purification and Analysis of Long RNA. Methods in Enzymology 2015; 558, 3-37.

305x   Identification of internal priming events in Lexogen's QuantSeq library preparation leads to accurate gene quantification and detection of 3' ends

Patrick Schagerl, Michael Ante, <u>Lukas Paul</u> and Andreas Tuerk

Lexogen GmbH, Campus Vienna Biocenter 5, Vienna, Austria

Accurate gene quantification and identification of 3'UTRs is important in many areas of biological research. Lexogen's QuantSeq library preparation provides an easy and fast protocol for generating highly strand-specific NGS libraries close to the 3' end of polyadenylated RNAs. The use of oligo(dT) primers in QuantSeq, however, also generates reads at long internal poly(A) stretches. Such reads can negatively affect the accuracy of end site detection and gene quantification. We, therefore, developed an internal priming filter (IPF) to remove reads associated with internal priming events. For this purpose, we investigate properties of the genome sequence and annotation around the read ends. In a window immediately downstream of the read end we count the number of A's, upstream we search for 3' motifs. We find the optimal cut-off for the number of A's by fitting a logistic regression model to sets of high-confidence internal priming and end sites having the same size ratio as internal priming and end sites in one chromosome. This ratio is determined by fitting a Gaussian mixture to the bimodal distribution of A-count frequencies. On the MAQC dataset the IPF retained 85.32% of the original reads in 3'UTRs, whereas only 5% and 3.68% were retained in 5'UTRs and introns, respectively. An increased concentration of reads at the gene ends can also be observed in coverage plots. Base content around priming sites after IPF showed the typical end site signature with an increased frequency of CA at the read end site, A upstream and GU/U downstream. Correlation between log fold changes of read counts and qPCR values increased after IPF from $R^2=0.7234$ to $R^2=0.8175$ suggesting superior accuracy in detecting differentially expressed genes. The described method is part of the QuantSeq pipeline including gene quantification and differential expression analysis which can be downloaded from the Lexogen website.

386x   Long read sequencing of nascent RNA in S. pombe reveals coupling among RNA processing events

Lydia Herzel, Korinna Straube, and <u>Karla M. Neugebauer</u>

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven USA

Pre-mRNA splicing is mediated by the spliceosome, a megadalton complex that assembles anew on each intron during transcription by RNA polymerase II (Pol II). We recently used single molecule RNA-Seq methods to determine the position of Pol II within budding yeast genes when the second step of splicing was completed[1]. Our data indicate that the spliceosome can act on the 3' splice site as soon as it emerges from Pol II. This leads to the prediction that splicing of multi-intron transcripts occurs in the order of their transcription, unless regulated by alternative splicing. We evaluated this in the fission yeast, S. pombe, which harbors >1000 genes with more than one constitutively spliced intron, by long-read sequencing of nascent transcripts. Similar proportions of 'in order' and 'not in order' splicing were observed, suggesting that splicing in order is not enforced and/or can be regulated. However, partially spliced transcripts were surprisingly rare when compared to a simulation based on individual intron splicing efficiencies determined from 75bp reads: 2.5-fold fewer partially spliced transcripts were observed (18%) than expected (45%). The largest fraction was fully spliced or unspliced, indicating that splicing of any given intron may depend on the splicing status of the other introns in the transcript. We show that fully unspliced transcripts failed to cleave at the polyA site, underwent transcriptional read-through at gene 3'ends, and were degraded by the exosome. Specific cases of retained individual introns were transported to the cytoplasm. We conclude that intron splicing and transcriptional read-through are dependent on the splicing status of neighboring introns, suggesting crosstalk among the spliceosomes and the 3'end processing machinery as they assemble during transcription.

[1] Carrillo Oesterreich, F., L. Herzel, K. Straube, K. Hujer, J. Howard, and K.M. Neugebauer. 2016. Splicing of nascent RNA coincides with intron exit from RNA polymerase II. Cell 165: 372-381.

ADD1 Combining asymmetric ^{13}C-labeling and isotopic filter/edit NOESY: rapid and logical RNA resonance assignment for identification of binding sites in RNA drug discovery

Regan M. LeBlanc[1,2], Andrew P. Longhini[1], Fardokht A. Abulwerdi[2], Stuart F.J. Le Grice[2], Theodore K. Dayie[1]
[1] Center for Biomolecular Structure and Organization, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, United States; [2] Basic Research Laboratory, National Cancer Institute, Frederick, MD, United States

RNA is an underutilized target for drug discovery. In fact, less than 1% of all approved drugs do not bind protein targets and more than 80% of drugs target only two classes of proteins: enzymes and receptors. RNA plays a critical role in essentially all aspects of biology including signaling, gene regulation, catalysis, and retroviral infection suggesting RNA is a promising target for drug discovery. However, less than 2% of the protein data bank structures comprise RNA yielding sparse structural information for rational drug design. The combined difficulties of crystallizing RNA for X-ray crystallography along with the rapid signal decay and spectral crowding associated with NMR have led to a stagnation in RNA structure publication. While half of the deposited RNA structures in the PDB were solved by NMR methods, the usefulness of NMR is still limited by the high cost of sample preparation and difficulties in resonance assignment. Here we propose a novel strategy for resonance assignment that combines new strategic ^{13}C labelling technologies with filter/edit type NOESY experiments to greatly reduce spectral complexity and crowding. This new strategy allowed us to assign important non-exchangeable resonances of proton and carbon (1',2',2,5,6, and 8) using only one sample and less than 24 hours of NMR instrument time for a 27 nt model RNA. The method was further extended to assigning a 6 nt bulge from a 61 nt viral RNA element justifying its use for wide range RNA chemical shift resonance assignment. Combining developments in fast pulsing techniques and non-uniform sampling methods, these improvements in resonance assignment methods have allowed us to implement chemical shift perturbation mapping as a preferred screening method for RNA drug discovery at greatly reduced costs.

ADD2 A case of polymorphism in a tetraloop due to a crystal packing interaction

Mélanie Meyer[1], Vincent Olieric[2], Jiro Kondo[3], <u>Henrik Nielsen</u>[4], Steinar Johansen[5], <u>Benoît Masquida</u>[6]
[1] Former address: Département de Génétique et de Génomique Structurales, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, Université de Strasbourg, Illkirch, France; [2] Swiss Light Source at Paul Scherrer Institute, Villigen, Switzerland; [3] Department of Materials and Life Sciences, Sophia University, Yotsuya Campus, Tokyo, Japan; [4] Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Copenhagen, Denmark; [5] Department of Medical Biology, University of Tromsø, Tromsø, Norway; [6] UMR 7156 GMGM - Centre National de la Recherche Scientifique, Université de Strasbourg, Strasbourg, France

To generate a single RNA conformer of the Lariat-Capping (LC) ribozyme, we engineered a circular permutation leading to the opening of the scissile bond and to the closure of the natural 5' and 3' extremities using a UUCG tetraloop [1,2]. The loop was supposed to have no effect on the whole structure. When we solved the structure, we noticed that the loop actually opened up in order to weave intimate interactions with a symmetry-related ribozyme molecule.

In order to check whether the conformation of the loop was due to the interaction, or if it opened up due to the design of our construct, we shortened the stem bearing the UUCG loop by one base-pair and solved the structure of this new construct. The crystal structure shows that in this slightly different context, the loop actually adopts the UUCG closed conformation that is observed in general.

We can conclude from these crystal structures that UUCG are indeed polymorphic and dynamic, and can serve interaction purposes, a function usually devoted predominantly to the GNRA tetraloops. This finding changes somehow the status of this kind of loops, which can be thought as a sensor as well as their cousins, the GNRA tetraloops. This finding may also orient future research to better characterize these loops to use them as crystallization modules among other applications.

1. Meyer, M.; Nielsen, H.; Olieric, V.; Roblin, P.; Johansen, S.D.; Westhof, E.; Masquida, B. (2014) Speciation of a group i intron into a lariat capping ribozyme. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 7659-7664.

2. Meyer, M.; Masquida, B. (2014) Cis-acting 5' hammerhead ribozyme optimization for in vitro transcription of highly structured rnas. *Methods in molecular biology*, *1086*, 21-40.

ADD3   NAD⁺-capped RNA and deNADding Enzymes in Human Cells

<u>Xinfu Jiao</u>[1], Selom K. Doamekpor[2], Jeremy G. Bird[3], Bryce E. Nickels[3], Liang Tong[2], Ronald P. Hart[1] and Megerditch Kiledjian[1]

[1]Dept. Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA; [2]Dept. Biological Sciences, Columbia University, New York, NY, USA; [3]Department of Genetics and Waksman Institute, Rutgers University, Piscataway, NJ, USA

Eukaryotic mRNAs are post-transcriptionally modified at the 5´ end by the addition of a m⁷G-cap to promote mRNA processing, translation and stability. The 5´ end could be further modified by additional methylations, including generation of a trimethylated, m2,2´7G cap or the production of Cap1 by methylation at the 2'O-ribose of the first nucleotide. More recently prokaryotic mRNAs were found to carry an unusual 5´ end modification consisting of a nicotinamide adenine dinucleotide (NAD⁺) moiety. This modification is proposed to protect the RNA from 5´ end decay by the bacterial RppH and RNaseE nucleases in vitro unless the NAD⁺ cap is hydrolyzed by the bacteria Nudix motif protein, NudC. We now demonstrate that mammalian mRNAs can also carry a 5´-end NAD⁺ cap and in contrast to the m⁷G cap, does not support translation but instead promotes mRNA decay. RNAs introduced into mammalian cells harboring a 5´-end NAD⁺ cap was less stable than RNAs with a m⁷G-cap or uncapped RNAs and failed to support translation any better than uncapped RNA. We further identify the DXO noncanonical decapping enzyme family of proteins as potent "deNADding" enzymes that efficiently remove NAD⁺ caps. Cocrystal structures of mammalian DXO and fungal Rai1 with 3´-NADP⁺ illuminate the molecular mechanism for how the deNADding reaction produces NAD⁺ and 5'-phosphate RNA. Removal of DXO from cells increases NAD⁺-capped mRNA levels and enables detection of NAD⁺-capped intronic snoRNAs, suggesting NAD⁺ caps can be added to 5´-processed termini by an unknown NAD-capping mechanism. Our findings establish NAD⁺ as an alternative mammalian RNA cap and DXO as a deNADding enzyme modulating cellular levels of NAD⁺-capped RNAs. Collectively, these data reveal mammalian RNAs can harbor a 5´-end modification distinct from the classical m⁷G cap that promotes, rather than inhibits, RNA decay.

497x   New Insights into RNA Structure and Dynamics of *Trypanosma brucei* Telomerase

Abhishek Dey[1,2], Rania Elbarki[1,2], Ranjodh Sindhu[3], Samantha Sanford[4], Niyati Jain[5], Blanton Tolbert[5], Bibo Li[3] and <u>Kausik Chakrabarti</u>[1,2]

[1]Dept. of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA; [2]Center for Nucleic Acid Science and Technology (CNAST), Carnegie Mellon University, Pittsburgh, PA, USA; [3]Department of Biological, Geo and Environmental Sciences, Cleveland State University, Cleveland, OH, USA; [4]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA; [5]Dept. of Chemistry, Case Western Reserve University, Cleveland, OH, USA

The control of cell proliferation is of central importance to the proper development of most organisms. Increased cell proliferation is essential to cancer and many lymphoproliferative diseases. Also, rapid and continuous cell proliferation is required by unicellular parasitic organisms to sustain long-term infection and cause disease pathogenesis. One of the major regulators of cellular proliferation in eukaryotes is a large RNA-protein enzyme complex, called Telomerase (1). Although much is known about telomerase regulation and function in higher eukaryotes, the intricate details of telomerase catalysis for chromosome end replication is poorly understood in pathogenic protists such as *Trypanosma sp.,* which represent the early branches eukaryotic phylogeny and cause devastating consequences on world health and economy with neurodegenerative and heart diseases. This is mainly because the RNA component of this enzyme, which provides the critical active site function by binding to the telomeric DNA substrate at the chromosome end, was unknown (2). Recently, we have identified the telomerase RNA in *T. brucei* (3) and provided genetic and biochemical evidence for its activity. Remarkably, this RNA has several unique features that suggest a mechanistically different process of telomerase-mediated telomere maintenance at the chromosome ends in *T. brucei* compared to yeast, mammalian, and Tetrahymena models. First, the RNA 'template' domain contains unique sequence permutations, suggesting an RNA-dependent, rather than a protein-dependent, anchoring interaction of the enzyme to its cognate substrate. Second, *T. brucei* and humans make identical telomeric DNA repeats (TTAGGG) using template domains that are vastly different in sequence composition, suggesting discrete

pathways of telomere replication. Third, many Telomerase RNA domains that interact with its integral *Reverse Transcriptase* protein component, are a composite of features from Telomerase RNAs of various organisms, however their functional significance remains unknown. Using RNA-SHAPE, NMR and functional assays, we have determined the structure and folding of the 'catalytic core' of this RNA molecule in *T.brucei* which provide in-depth understandings of the mechanistic aspects underlying RNA structure formation. These data should further our knowledge on understanding novel aspects of telomerase regulation in deep-branching eukaryotes.

References:
1. Blackburn EH, Greider CW, Szostak JW. 2006. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med.* 12(10):1133-8.
2. Muñoz DP, Collins K. 2004. Biochemical properties of *Trypanosoma cruzi* telomerase. *Nucleic Acids Res.* 32(17): 5214-22.
3. Sandhu R, Sanford S, Basu S, Park M, Pandya UM, Li B, Chakrabarti K. 2013. A trans-spliced telomerase RNA dictates telomere synthesis in *Trypanosoma brucei*. Cell Research 23:537-551.

Added in topic area <u>*RNAs in Disease*</u> *(position between 567 and 568)*

ADD4   The serotonin receptor 2C in pituitary is deregulated in Prader-Willi syndrome

<u>Justin Welden</u>[1], Zhaiyi Zhang[1], Tim Wells[2] and Stefan Stamm[1]
[1]University of Kentucky, Lexington, KY, USA; [2]Cardiff University, Cardiff, Wales, UK

The serotonin receptor 2C is a seven transmembrane receptor regulating mood and appetite in the central nervous system. Its pre-mRNA undergoes alternative splicing of exonVb creating two 5HT2C isoforms: RNA1 that encodes a truncated receptor and RNA2 that encodes a full-length receptor. Due to additional editing, the 5HT2C generates a total of 33 mRNAs encoding 25 proteins. ExonVb inclusion is promoted by a snoRNA, SNORD115, likely by changing the pre-mRNA structure around exonVb. SNORD115 is missing in Prader-Willi syndrome, a genetic cause for obesity and intellectual disability. The truncated receptor encoded by RNA1 heterodimerizes with the full-length receptor (RNA2), leading to an internalization of the full-length receptor stopping 5HT2C signaling. Thus, the isoform ratio between RNA1 and RNA2 controlled by alternative splicing regulates the activity of the 5HT2C, and possibly other receptors, such as the ghrelin receptor (GHSR1) due to heterodimerization.

Short stature caused by low growth hormone levels is a characteristic feature of PWS. Growth hormone is secreted after ghrelin receptor activation from the pituitary. Since growth hormone deficiency is central to PWS, we tested expression of the 5HT2C in pituitary and detected expression of RNA1 and RNA2 in mice, human and rat pituitaries. In addition, SNORD115 that regulates alternative splicing of 5HT2C is expressed as well, suggesting a regulation of the 5HT2C isoforms through SNORD115 in pituitary. We therefore analyzed mice that lack SNORD115 and found a 1.6 fold increase of the RNA1/RNA2 ratio, concomitant with the loss of SNORD115.

We next tested the effect of food withdrawal on the 5HT2C isoforms and found an almost complete loss of RNA1 after 48 hrs of fasting, suggesting that 5HT2C isoform ratios are under control of physiological stimuli. We previously developed an oligonucleotide [1] that promotes exonVb inclusion, similar to SNORD115 and found that after tail vein injection, this oligo strongly promotes exonVb inclusion in pituitary, further demonstrating a regulation of the RNA1/RNA2 ratio.

Our data suggest that the 5HT2C plays, so far, an unknown role in pituitary, possibly regulating growth hormone release through heterodimerization with other receptors, such as the GHSR1a. It is likely deregulated in PWS, contributing to the hormonal distortions characteristic for the disease.

1.Zhang Z, Shen M, Gresch P, Ghamari-Langroudi M, Rabchevsky AG, Emeson RB, Stamm S. Oligonucleotide-induced alternative splicing of serotonin 2C receptor reduces food intake. EMBO molecular medicine. 2016;8(8):878-94.PubMed PMID: 27406820.

ADD5   Modeling retinitis pigmentosa - investigation of changes in pre-mRNA binding and alternative splicing to understand the onset and cause of photoreceptor degeneration

Michaela Krausova[1], Jan Prochazka[2], Radislav Sedlacek[2], and David Stanek[1]
[1] Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic; [2]Czech Centre for Phenogenomics, BIOCEV, Vestec, Czech Republic

Background and aims: Germline mutations in genes encoding components of the pre-mRNA splicing machinery underlie development of retinitis pigmentosa (RP), a degenerative disease characterized by progressive loss of photoreceptor cells. Given the eye-exclusive manifestation, the phenotype suggests enhanced sensitivity of ocular cell populations for proper spliceosome assembly and/or its outcomes. Using a blend of *in vivo* and *in vitro* models, we aim to specifically explore the molecular principles by which aberrant splicing events contribute to retinal malfunction.

Methods and Results: We used TALEN and CRISPR/Cas9 genome editing tools to establish novel murine strains harboring the RP-causative substitution mutations Prpf3 Thr494Met, Prpf8 Tyr2334Asn, and moreover Brr2 Ser1087Leu. Secondary to correct SNPs, we also gained *Prpf3* and *Prpf8* null alleles by small Indel events; the latter strains serve comparison with presumptive loss-of-function RP mutations. In aging mice, the retinal condition is being repeatedly assessed using noninvasive optical coherence tomography and electroretinography, with histopathological analysis performed in parallel cohorts. In addition, physiological as well as diseased retinal transcriptome and the related splicing patterns will be examined by deep sequencing of the RP-mutant and wild-type counterpart retinae.

In parallel, we used corresponding nuclease editing systems to introduce tagged variants of RP-relevant splicing factors to cultured, human retina-derived cells RPE-1. Here, we will employ the iCLIP technique to explore, whether altered magnitude of binding and/or specificity in site recognition may lead to aberrant splicing events.

Conclusions: We have established an innovative array of *in vivo* and *in vitro* tools to scrutinize the splicing execution in RP-compromised retinal cells. Using transcriptome-wide data in combination with protein-RNA interaction assays, we hope to dissect how inappropriate splicing corrupts the fitness of RP-stricken retinal cell populations.

ADD6   An Atomic Structure of the Human Spliceosome

Xiaofeng Zhang[1,3], Chuangye Yan[1,3], Jing Hang[1,3], Lorenzo I. Finci[1], Jianlin Lei[1,2], and Yigong Shi[1,4]
[1]Beijing Advanced Innovation Center for Structural Biology, Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, China; [2]Technology Center for Protein Sciences, Ministry of Education Key Laboratory of Protein Sciences, School of Life Sciences, Tsinghua University, Beijing, China

Mechanistic understanding of pre-mRNA splicing requires detailed structural information on various states of the spliceosome. Here we report the cryo-EM structure of the human spliceosome just prior to exon ligation (the C* complex) at an average resolution of 3.76 Å. The local resolution for about 20 components in the core of the spliceosome reaches 3.0-3.5 Å, allowing identification of amino acid side chains and assignment of RNA nucleotides. Compared to the yeast C* complex, a notable difference is formation of a considerably longer duplex between U6 snRNA and the 5'SS and ensuing sequences of the intron in the human C* complex. The splicing factor Prp17 stabilizes the active site conformation by interacting with multiple proteins. The step II factor Slu7 adopts an extended conformation, binds Prp8 and Cwc22, and is poised for selection of the 3'-splice site. Remarkably, the intron lariat traverses through a positively charged central channel of RBM22; this unusual organization suggests mechanisms of intron recruitment, confinement, and release. The protein PRKRIP1 forms a 100-Å α-helix linking the distant U2 snRNP to the catalytic center, suggesting an important role in splicing reaction. A 35-residue fragment of the ATPase/helicase Prp22 latches onto Prp8. The distance between the 3'-end of the intron lariat and the RNA binding site of Prp22 spans approximately 100 Å, which requires a minimal of 15 nucleotides in their fully extended conformation. The quaternary exon junction complex (EJC) recognizes upstream 5'-exon sequences and associates with Cwc22 and the GTPase Snu114. These structural features reveal important mechanistic insights into exon ligation.

Added in topic area <u>Translational Regulation</u> (in the space vacated by withdrawn 676)

676x   RNA-binding proteins influence stem cells' fate decisions

<u>Monika Gaik</u>, *Florentina Freynschlag, Sebastian Glatt*

Max Planck Research Group at Malopolska Centre of Biotechnology, Jagiellonian University in Cracow, Poland

Post-transcriptional regulation of gene expression plays an important role during fundamental physiological processes in eukaryotes. The decision whether stem cells will keep their self-renewal potency or switch into differentiation pathway occurs at each stem cell division and has to be under strict control by numerous stem-cell maintenance and differentiation factors. Fruit fly early germline development has been used as a powerful model for studying translational control. The precise translational regulation employs diverse RNA-binding proteins (RBPs) and chemical modifications which have a crucial effect on adjusting the translation and protein folding dynamics. By forming ribonucleoproteins (RNPs) upon binding to specific motifs of mRNA transcripts, RBPs act as regulatory elements and provide additional level of translational control through RNPs' dynamic and constant remodeling events. Any defects in this regulation may lead to continuous proliferation of stem cells resulting in carcinogenesis and infertility.

To further our understanding of how mRNA transcripts are efficiently "fine-tuned" by RNPs assembly and control protein translation, we have chosen a novel macromolecular complex involved in translational repression during stem cells switch to differentiation pathway. Translational repression of particular transcripts relies on protein-RNA and protein-protein interactions. Upon binding of RBPs to U-rich RNA elements present in the 3' <u>un</u>translated <u>re</u>gion (3' UTR) of mRNA, the synthesis of stem cells maintenance factors is inhibited and differentiation events occur. Our knowledge on how RBPs orchestrate mRNA binding and subsequently control translation remains limited.

To study regulatory mechanisms of protein synthesis we combine protein biochemistry and biophysics with structural biology. By determining structural characteristic of RBPs interactions with molecular partners (RNA and other proteins) we plan to identify exact protein domains and RNA motifs important for translational regulation during oogenesis. To address the hierarchy of RNPs assembly events within the repressive complex we perform protein interaction analyses and competition assays of reconstituted sub-complexes. This approach allows to determine if RNA-binding proteins bind cooperatively or stepwise to the regulatory motifs on mRNA transcripts. Through gaining details about specific inhibitory protein-RNA interactions and RNPs formation we aim to deepen our knowledge of important regulatory processes that determine cell fate.