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Non-coding and Regulatory RNAs

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157-B Overexpression of small noncoding RNAs and its effects on biofilm-related phenotypes in *Escherichia coli**Geunu Bak*¹, *Kwang-sun Kim*², *Younghoon Lee*¹¹Department of Chemistry, KAIST, Daejeon 305-701, Korea; ²Systems and Synthetic Biology Research Center, KRIBB, Daejeon 305-806, Korea

It is undoubted that noncoding RNA has been deeply involved in various metabolic pathways in organisms ranging from bacteria to mammals many different organisms. Although nearly 100 small noncoding RNAs (sRNAs) have been experimentally verified in *E. coli*, knowledge about their roles has been continuously expanded through identification of functions of newly identified sRNAs as well as new roles of previously known sRNAs. Recently, roles of sRNAs in bacterial group behavior or pathogenesis have received a great deal of attention. Biofilm, a surface-bound and self-aggregate structure of bacteria embedded within extracellular polymeric substances, is one of their important group behaviors for survival in varying environmental challenges. In this study, a plasmid library expressing 99 experimentally verified *E. coli* sRNAs was constructed on IPTG-inducible RNA expression vector pHMB1/2. Using this sRNA-expressing library, changes of biofilm related phenotypes upon overexpression of each sRNA were examined. Especially flagella-based motility and cell surface appendages were analyzed in details.

160-B An RNA Degradation Machine Sculpted by Ro Autoantigen and Noncoding RNA*Xinguo Chen*¹, *David Taylor*³, *Casey Fowler*², *Soyeong Sim*¹, *Jorge Galan*², *Hong-Wei Wang*³, *Sandra Wolin*¹¹Department of Cell Biology, Yale University; ²Department of Microbial Pathogenesis, Yale University; ³Department of Molecular Biophysics and Biochemistry, Yale University

Although the functions of many RNA-protein complexes (RNPs) are well understood, the roles of others are still being elucidated. One class whose function remains under investigation is the Ro class of RNPs. The major protein component, the ring-shaped Ro 60 kDa autoantigen (Ro60), is present in many animal cells and also in ~5% of sequenced bacterial genomes. In all characterized species, Ro60 binds ~100 nt noncoding RNAs (ncRNAs) of unknown function called Y RNAs. Because Ro60 also binds misfolded rRNAs and snRNAs in some animal cell nuclei, it is proposed to function in ncRNA surveillance. In the only bacterium in which Ro60 has been characterized, *Deinococcus radiodurans*, the ortholog Rsr functions with 3' to 5' exoribonucleases during some types of environmental stress. Specifically, Rsr and the exoribonucleases RNase II and RNase PH are required for efficient 23S rRNA maturation during heat stress. In addition, Rsr and the exoribonuclease polynucleotide phosphorylase (PNPase) are involved in rRNA degradation during stationary phase.

To understand how a Ro60 protein can influence the function of an exoribonuclease, we purified the Rsr/PNPase complex from *D. radiodurans* and examined its composition, molecular architecture and activity. We discovered that Y RNA tethers Rsr to PNPase to form an RNA degradation machine. Single particle electron microscopy (EM), followed by docking Ro and PNPase atomic structures into the three-dimensional reconstruction, revealed a double ring architecture, suggesting that Rsr channels RNA into the PNPase cavity for degradation. Biochemical experiments revealed that Rsr and Y RNA specialize PNPase for degrading structured RNA. Specifically, the Rsr/Y RNA/PNPase RNP is more effective than PNPase alone in degrading stemloop-containing RNAs, but is less active than PNPase on single-stranded RNA. These studies identify a role for Y RNA and also show that ncRNA, by tethering a protein cofactor, can alter the substrate specificity of an enzyme.

To determine if the role we identified for Rsr and Y RNA in *D. radiodurans* could be conserved in other bacteria, we examined *Salmonella* Typhimurium. We found that *S. Typhimurium* Rsr associates with two novel ncRNAs encoded 3' to *rsr*. One ncRNA, which we call Yr1A, appears to be highly conserved, as potential orthologs are encoded adjacent to the Rsr ortholog in more than 100 bacterial genomes. Notably, by performing immunoaffinity experiments in *S. Typhimurium*, followed by glycerol gradient sedimentation, we found that Rsr and Yr1A also associate with PNPase in this bacterium. Thus, assisting degradation of structured RNAs is likely to be a conserved role of bacterial Ro RNPs.

163-B The microRNA pathway mediates expression of yolk lipoproteins in the *Caenorhabditis elegans* intestine*Robert Downen*¹, *Gary Ruvkun*¹¹Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.

In the nematode *Caenorhabditis elegans*, yolk lipoproteins (VIT proteins) are synthesized in the hermaphrodite intestine, secreted, and internalized by the oocytes where their associated lipids provide nourishment throughout embryogenesis [1]. Here, we show that the microRNA (miRNA) pathway is required for proper production of the VIT lipoproteins, and consequently, for proper embryonic development. An expression analysis of the *vit* genes revealed that the miRNA pathway functions upstream of *vit* transcription. Moreover, two miRNAs, *let-7* and *lin-4*, are specifically required for proper endogenous *vit* gene expression, as well as activation of a GFP-based *vit-2* transcriptional reporter. Using the *vit-2* reporter, we have identified two gene inactivations that suppress the effects of a *let-7* mutation and partially restore *vit-2* gene expression. These two genes, *lin-14* and *lin-41*, are known targets of the *let-7* and/or *lin-4* miRNAs. We are currently investigating whether *let-7* and *lin-4* function within the intestine to control *vit* gene expression, or alternatively, whether they act in surrounding tissues to regulate intestinal development.

1. Kimble J, Sharrock WJ (1983) Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. Dev Biol 96: 189–196.

166-B Novel RNA Structures Controlling Ribosomal Protein Biosynthesis in *E. coli* and BeyondYang Fu¹, Michelle Meyer¹¹Boston College

The control of ribosomal protein biosynthesis is tightly coordinated in eubacteria. In *E. coli* there are over 10 different RNA structures that act to autogenous regulate over half of the ribosomal protein genes. Each of these cis-regulatory RNAs interacts with a specific ribosomal protein to inhibit transcription or translation of an entire operon encoding multiple ribosomal proteins. Despite the important role these RNAs play in regulating the biosynthesis of an essential process, our recent work shows that many of them appear to be narrowly distributed to a few groups of closely related bacteria. This, in combination with the discovery of several putative RNA structures associated with ribosomal protein genes in different phyla of bacteria, suggests that there are numerous RNA structures responsible for regulation of ribosomal protein biosynthesis that remain to be discovered.

Using comparative genomics we identified several RNA structures associated with ribosomal protein operons, including one that precedes ribosomal protein genes *rpsF* and *rpsR*, encoding ribosomal proteins S6 and S18 respectively. This RNA structure is widely distributed to many bacterial phyla (it is found in both *E. coli* and *B. subtilis*), and overlaps a potential Shine-Dalgarno sequence in many organisms. Using *in vitro* assays we confirmed that this RNA interacts specifically with S18, and with an S6:S18 dimer suggesting that the RNA is a novel autogenous regulatory element responsible for coordinating the levels of ribosomal proteins in bacteria. This work shows that comparative genomic methodologies applied so successfully to identify riboswitch candidates may also be applied to the discovery of RNA regulators for ribosomal protein biosynthesis that may have been overlooked using previous approaches.

169-B RNA annealing activity of Hfq is sensitively modulated by various physical parameters*Wonseok Hwang*¹, *Véronique Arluison*³, *Sungchul Hohng*²

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Bacteria regulate their gene expression level in response to various environmental stresses. One of their regulation methods is small non-coding RNAs (sRNA) which acts at the post-transcriptional level by base-pairing with their target mRNA. For example, *E. coli* DsrA sRNA is expressed under cold shock and up-regulates RpoS (Sigma-S) transcription factor by base-pairing with *rpoS* mRNA. It is well known that this sRNA regulation process requires the RNA chaperone Hfq for efficient RNA duplex formation. However, how the RNA annealing activity of Hfq is modulated under varying physical parameters due to various environmental stresses is ill-understood. Here we investigate how the annealing activity of Hfq is modulated by various physical parameters by using single-molecule FRET assays. We use dye-labeled synthetic RNA fragments obtained from *E. coli* DsrA sRNA and from its target mRNA, *rpoS*. Our results show that low temperature increases the annealing efficiency by accelerating Hfq dissociation from DsrA:*rpoS*:Hfq ternary complex. Relative ratio between RNA and protein is also important as an excess Hfq lowers annealing efficiency by inhibiting DsrA:*rpoS*:Hfq ternary complex formation. The concentration of various salts greatly influences the annealing efficiency by increasing the turnover number of Hfq. Finally, the molecular crowding, which Hfq may encounter *in vivo*, enhances the annealing efficiency by increasing effective concentration of RNA and Hfq. Collectively, our results suggest that the activity of Hfq can be sensitively modulated by various physical parameters found *in vivo*.

172-B Long ncRNA NEAT1-dependent SFPQ relocation between nuclear body paraspeckle and gene promoter region mediates the transcription of IL8 gene in immune response

Katsutoshi Imamura¹, Gen Akizuki⁷, Naoto Imamachi⁷, Michiko Kumakura², Atsushi Kawaguchi², Kyosuke Nagata², Akihisa Kato⁴, Yasushi Kawaguchi⁴, Hiroki Sato⁵, Misako Yoneda⁵, Chieko Kai³, Tetsushi Yada³, Shinichi Nakagawa⁸, Kiyomi Kaneki⁶, Kenji Inoue⁶, Tatsuhiko Kodama⁶, Youichiro Wada⁶, Kazuhisa Sekimizu¹, Nobuyoshi Akimitsu⁷

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Whole transcriptome analyses have revealed that novel classes of non-protein-coding transcripts, designated long noncoding RNAs (lncRNAs), were transcribed from mammalian genome. As the ratio of non-coding to protein-coding genomic regions increased as a function of developmental complexity, it has been assumed that the roles of lncRNAs transcribed from non-coding genomic regions are important to understand the genome function in higher organisms. NEAT1, a nuclear lncRNA, is essential for the formation of paraspeckle, one of nuclear bodies. NEAT1 consists two isoforms, 3.7-kb NEAT1v1 and 23-kb NEAT1v2, and the ratio between NEAT1v1 and NEAT1v2 are regulated by alternative 3'-end processing. NEAT1v2 but not NEAT1v1 is potent for the formation of paraspeckle, where NEAT1v2 interacts with splicing factor, proline/glutamine-rich (SFPQ) protein. However, the function of paraspeckles and NEAT1v2 are largely unknown.

We found that the levels of NEAT1v2 and IL8 mRNA were raised by poly I:C transfection. Poly I:C transfection induced excessive formation of paraspeckles without altered expression level of SFPQ. NEAT1 knock down decreased polyI:C-induced IL8 mRNA level. Conversely, solo overexpression of NEAT1v2 increased IL8 mRNA level and excessive formation of paraspeckles. These results suggest that NEAT1v2 regulates the expression of IL8 mRNA under polyI:C stimulation. Next, we investigated the mechanism how NEAT1v2 regulates IL8 mRNA expression. SFPQ knock down increased the *IL8* promoter activity as well as IL8 mRNA level, demonstrating that SFPQ repressed the transcription of IL8 mRNA. To test whether SFPQ directly binds the promoter region of *IL8* or not, we performed chromatin immunoprecipitation. Quantitative PCR analysis of the purified immunoprecipitated DNA binding with endogenous SFPQ showed that SFPQ bound the *IL8* promoter region in naive cells. In addition, either poly I:C stimulation or solo overexpression of NEAT1v2 decreased binding of SFPQ on the *IL8* promoter. Finally, we tested whether virus infection induced up-regulation of NEAT1v2. Influenza virus or herpes simplex virus 1 (HSV-1) but not measles virus infection induced NEAT1v2. We confirmed that HSV-1 infection induced excessive formation of paraspeckles. These findings suggested that viral infection increases NEAT1v2 transcription and induces excessive formation of paraspeckles where SFPQ is relocated from promoter region of *IL8*, consequently, up-regulates IL8. We propose that paraspeckles function as a "molecular absorber" of paraspeckle proteins to regulate gene expression in response to stimuli.

175-B Recognition of brain cytoplasmic 200 RNA by a human anti-RNA antibody*Euihan Jung¹, Jungmin Lee¹, Hyo Jeong Hong², Insoo Park³, Younghoon Lee¹***¹Department of Chemistry, KAIST, Daejeon 305-701, Korea; ²Department of Systems Immunology, College of Biomedical Science, Kangwon National University, Chuncheon 200-701, Korea; ³Molecular Imaging & Therapy Branch, National Cancer Center, Goyang-si 410-769, Korea**

It is known that diverse functional RNAs participate in a wide range of cellular processes. RNA structure plays an important role in their functions by themselves and as complex forms with proteins or ligands. Therefore, monitoring RNA conformation in the cell is essential for understanding their functional mechanisms, but any appropriate method is not established yet. Although hybridization is a general method used for analyzing specific RNA molecules through their base complementarity, it would be difficult to apply for monitoring RNA conformation because the hybridization requires partially denatured conditions, which could disturb their structural integrity. In the current study, we developed an efficient strategy for screening human monoclonal antibodies binding to RNA from a naïve antigen binding fragment (Fab) combinatorial phage library, using brain cytoplasmic 200 (BC200) RNA as a bait. BC200 RNA is a neuron-specific non-coding RNA that operates as a translational modulator, implicated in the inhibition of local synaptodendritic protein synthesis, in human cells. The neuron-specific BC200 RNA has been reported to be also expressed at high levels in invasive carcinomas than benign tumors of the breast. We biopanned a large human Fab combinatorial phage library (5×10^{11} recombinants), and isolated two antibodies that recognize BC200 RNA, and one of them was selected for further affinity maturation by modifying residues in LCD3. The best binding antibody, MabBC200-A3, binds specifically to the two regions of BC200 RNA (residues 76 to 85 and 96 to 104) with dissociation constant of about 7 nM. In the secondary structure model, the two regions were separated by approximately one-half helix-turn so that they could be placed in the same surface for interaction with the antibody. Various breast cancer cell lines were examined for their BC200 RNA expression using conventional hybridization, and BC200 RNAs expressed in those cells were analyzed with MabBC200-A3. MabBC200-A3 discriminates BC200 RNA from homologous 7SL RNA in purified total cellular RNA. The amount of BC200 RNA recognized by MabBC200-A3 in the purified total RNA pool was proportional to the cellular level of BC200 RNA, but the amount of antibody-recognizable BC200 RNA in the cell was not, suggesting that BC200 RNA can exist in distinct states in the cell. Our data show that anti-RNA antibody can provide a novel tool for RNA-detecting and analyzing that hybridization cannot provide.

178-B Roles for roX RNA and the RNA helicase MLE in the assembly of the dosage compensation complex in *Drosophila*

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Dosage compensation in *Drosophila melanogaster* involves the transcriptional activation of genes on the single male X chromosome to match their expression levels to those in females, where both X chromosomes are transcribed.

The regulatory dosage compensation complex (MSL-DCC) consists of five so-called male-specific lethal (MSL) proteins and two long, non-coding RNA, *roX1* and *roX2*, whose function is enigmatic. RNA helicase maleless (MLE) is among the MSL subunits. It is crucial for dosage compensation, but its role remains unclear. Due to its unwinding activity and its multiple RNA-binding domains, MLE is well suited to play a key role in *roX* functionality and may be involved in regulating the association of *roX* with the MSL components. Up to now, biochemical analyses had found that MLE associates only weakly with the other MSL proteins in nuclear extracts and *in vitro* binding studies failed to reveal any specific interaction of MLE with *roX* RNA.

We report on the results of our recent efforts to characterize the interactions of MLE with roX RNA, combining by RNA pull-down assays, RNA 2D structure analysis and footprint experiments. We found that MLE is able to recognize specific aspects of *roX* RNA and to change its 2D structure to form that is competent to initiate MSL assembly. Our data strongly suggest that the ATP-dependent remodeling of *roX* RNA by MLE may be rate limiting for MSL-DCC assembly.

181-B Fission yeast Cactin silences chromosome ends and retrotransposons and links heterochromatin establishment to telomere length regulation*Martin Moravec¹, Luca E Lorenzi¹, Amadou Bah¹, Harry Wischniewski¹, Marco Santagostino¹, Claus M Azzalin¹*¹**Institute of Biochemistry, ETH Zürich, Zürich, CH-8093, Switzerland**

Eukaryotic telomeres are transcribed by RNA polymerase II into diverse long non-coding RNA (ncRNA) molecules forming the telomeric transcriptome. Among these ncRNA species, telomeric repeat-containing RNA (TERRA) is conserved in eukaryotes and remains associated to telomeres post-transcriptionally, suggesting that TERRA is an evolutionarily conserved integral component of telomeric heterochromatin. Still, the functions and the mechanisms of regulation of the telomeric transcriptome remain enigmatic. We have screened a complete *Schizosaccharomyces pombe* gene-deletion collection and identified several mutants with increased TERRA cellular levels. One of these strains is deleted for the gene encoding 'Fission yeast Cactin-like protein 1' (Fyc1), the *S. pombe* member of the eukaryotic Cactin protein family, which comprises poorly characterized polypeptides possibly involved in cell cycle progression and cell growth. Consistently, *fyc1Δ* yeasts grow slower compared to wild type counterparts and are sensitive to a cold environment. Fyc1 binds telomeric and subtelomeric sequences at low levels *in vivo*, and *fyc1Δ* mutants accumulate subtelomeric and telomeric RNA and fail to silence subtelomeric reporter genes. Thus, Fyc1 is necessary to silence chromosome ends and to establish telomere position effect. In absence of Fyc1, telomere elongation by telomerase is exaggerated and the telomeric G-overhang is longer, suggesting that telomere transcription may stimulate telomerase-mediated telomere elongation. Intriguingly, cells deleted for *fyc1+* also accumulate RNA deriving from all Tf2 retrotransposons and numerous solo LTR elements scattered throughout the genome, implying that Fyc1 promotes concomitant silencing of telomeres and elements of retroviral origin. Mechanistically, Fyc1 sustains establishment of heterochromatin at telomeres and retrotransposon-containing loci by restricting the levels of acetylated histone H3 at lysine 9 (H3K9) and, conversely, promoting accumulation of trimethylated H3K9. Our findings reveal that Fyc1 is a novel regulator of telomeric heterochromatin establishment and telomere length maintenance, and link silencing of telomeres and retrotransposons. We are investigating whether and to what extent the different phenotypes observed in *fyc1Δ* cells are causally linked.

184-B Malignant Transformation Changes Packaging and Targeting of Extracellular MicroRNAs*Jaime Palma*¹, *William Pearce*¹, *Mallory Havens*², *Michelle Hastings*², *Dominik Duelli*¹¹Department of Cellular and Molecular Pharmacology, The Chicago Medical School at Rosalind Franklin University, North Chicago, IL, 60064 USA; ²Department of Cell Biology, The Chicago Medical School at Rosalind Franklin University, North Chicago, IL, 60064 USA

Cells regulate each other remotely in many ways. An emerging mechanism is the exchange of microRNA (miRNA) packaged into membrane-coated vesicles. We reported that several cellular processes associated with malignant transformation change the export of extracellular miRNAs (ex-miRs) by affecting whether a particular miRNA species is released selectively or retained by the cell. We here describe the basis of selective ex-miR release and transfer to target cells.

We show that ex-miRs are packaged mutually exclusively in different carriers released from breast cancer cells. miR-451, which is selectively released from transformed cells associates with exosomes, miR-1246 with nucleosomes, and neutrally released miR-16 associates with unconventional "L-" exosomes. In contrast, normal cells release these ex-miRs in a single type of vesicle. Stresses upon the donor cells, including DNA damage, affect the export of miR-16. We further demonstrate that uptake of ex-miRs and its consequences are cell-type and carrier-type specific. For example, T-cells accumulate ex-miR-16 and ex-miR-451, while megakaryocytes internalize miR-16 but not miR-451. The received miR-16 represses BCL2 in T-cells, triggering apoptosis, but causes cell cycle arrest in megakaryocytes. Finally, monocytes do not acquire miR-16 or miR-451 from the extracellular environment, and the cells differentiate.

Malignant transformation induces *de novo* extracellular vesicles, into which some ex-miRs are assorted mutually exclusively. This separation is key in determining the cell-type specific delivery of extravesicular cargo, and can explain how ex-miRs can simultaneously activate cancer-promoting cells and block anti-cancer cells. This relay is dynamic, as changes in the ex-miR population due to DNA damage in the cancer cell of origin is relayed to other cells and affects their function. We propose a model wherein ex-miR-cell signaling occurs similar to virus-cell interactions, including the need for ligand-receptor interactions, and subsequent cellular trafficking.

187-B Discovery of Hfq-binding nanoRNAs in *Escherichia coli**Jennifer Patterson*¹, *Shugeng Cao*², *Jon Clardy*², *Cameron Mura*¹¹University of Virginia; ²Harvard University

RNA-based regulation enables exquisite control over the extent and timing of gene expression, thereby enabling bacteria to rapidly respond to their environment. The bacterial host factor Hfq acts as a post-transcriptional regulator of such changes in gene expression. This cellular activity likely stems from the ability of Hfq to function as a generic RNA chaperone. Previous work has found that Hfq preferentially interacts with A/U-rich RNAs. In this study, we discovered an Hfq that interacts with short U/C-rich nanoRNAs, when recombinantly expressed in *Escherichia coli*. These Hfq-binding nanoRNAs interact with high affinities (nanomolar-scale) and feature specific RNA end-chemistries (5'-monophosphate, 3'-hydroxyl). These unanticipated Hfq·nanoRNA associations may represent a novel mechanism by which Hfq and Hfq·sRNA complexes modulate RNA-based regulatory circuits *in vivo*.

190-B Integrated genome-wide in silico and capture array approach discovers a large spectrum of novel structured RNAs associated to regulatory elements

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Computational predictions and high-throughput sequencing techniques, such as RNA-seq, have recently given rise to the discovery of many non-coding RNAs (ncRNAs). In silico methods have in particular focused on predicting RNA structure, which is a functional characteristic of many ncRNAs. However, these screens have primarily searched in regions of high evolutionary sequence conservation and lack of large scale experimental follow up analysis.

To overcome these limitations, we introduce a genome-wide (on all regions covered by MAF blocks) in silico screen for ncRNAs based on structural RNA alignments (of corresponding sequence from vertebrates) in direct combination with the design of a capture array for expression analysis. The in silico screen resulted in ~600,000 highly structured candidate regions in the human genome of an estimated false discovery rate of 26%. For the top ~60,000 regions the FDR is less than 10%. We also predict a genome-wide coverage by conserved RNA structures of around 14%. The identified regions are primarily intergenic and are enriched for untranslated regions (UTRs) in mRNAs. We observe good overlap to known ncRNAs and recently identified long ncRNAs. The study reveals that structured RNAs are most adequately predicted from structural alignments and, interestingly, comprise RNA structures of down to 20% sequence identity.

Functional indications of the conserved RNA structures are given by their location adjacent to many regulatory features such as transcription factor binding sites and the evidence for their negative selection. To provide further confidence on the in silico predictions we performed the first large-scale experimental analysis of structured ncRNA candidates by probe design of ~80,000 structured candidate regions. The designed capture array reveals the expression of ~8,000 of these regions. The chosen strategy of RNA capture array and high-throughput sequencing found a number of low expressed ncRNAs and RNA structures in extended UTRs which have not been found by ordinary RNA-seq experiments. Further experiments (qPCR, RACE and structure probing) in human and mouse support our hypothesis of transcripts weakly conserved in sequence but with a highly conserved RNA structure.

193-B Uncovering novel microRNAs involved in homeostatic plasticity

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Synaptic scaling is a homeostatic mechanism responsible for the adjustment of the overall synaptic strength in a neuron's synapses to a set point, in order to stabilize neuronal firing. Dendritic protein synthesis is crucial for synaptic scaling but the mechanisms that regulate the de-repression of mRNAs are still unclear. MicroRNAs, well known posttranscriptional regulators, are modulated upon changes in neuronal activity, which indicates that miRNAs may have a role in the regulation of homeostatic plasticity. Our main goal is to unveil novel miRNA players during synaptic scaling and to further investigate their role in this form of plasticity.

We performed a gene expression microarray analysis of rat hippocampal neurons under chronic blockade of activity and submitted the results to biological interpretation using the GoMiner tool. Several gene categories, relevant for synaptic events, were selected and miRNA target sites were predicted for those genes using the following algorithms: miRanda, TargetScanS and MirTarget2. Focusing on a restricted group of altered genes with a crucial role in synaptic scaling and/or in synaptic function, we identified a group of 18 predicted miRNAs, and performed a screening panel for their expression levels in primary cultures of rat hippocampal neurons subjected to synaptic scaling up conditions. This analysis revealed that several miRNAs present altered expression in scaling up conditions and therefore suggests an important role for these miRNAs in synaptic scaling mechanisms.

196-B A link between long intervening noncoding RNAs and microRNA regulation*Igor Ulitsky¹, Alena Shkumatava¹, Hazel Sive¹, David Bartel²***¹Whitehead Institute; ²Whitehead Institute for Biomedical Research, Howard Hughes Medical Institute and Department of Biology, MIT**

Thousands of long intervening noncoding RNAs (lincRNAs) have been identified in mammals. To better understand the evolution and functions of these enigmatic RNAs, we identified more than 550 distinct lincRNAs in zebrafish. Although these shared many characteristics with mammalian lincRNAs, only 29 had detectable sequence similarity with putative mammalian orthologs, typically restricted to a single short region of high conservation. Other lincRNAs had conserved genomic locations without detectable sequence conservation. Morpholinos targeting conserved regions of two zebrafish lincRNAs caused developmental defects. Morpholinos targeting splice sites caused the same defects and were rescued by adding either the mature lincRNA or its human or mouse ortholog. In one of the lincRNAs, called *cyrano*, the conserved region extensively pairs to miR-7, and this pairing has been essentially unchanged since the dawn of vertebrates. Our experiments confirm that the conserved site is a bona fide microRNA target site that is bound by argonaute proteins and confers regulation by miR-7. This regulation is part of a larger network of miRNA-lincRNA regulations, which is revealed by multiple types of experimental evidence. We also demonstrate how direct comparison of lincRNA sequences across distant species can uncover miRNA complementary sites and other conserved elements that are missed in whole-genome alignments.

199-B HEN1-directed labeling of small non-coding RNAs

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MicroRNAs and siRNAs are 21-25 nucleotides long non-coding RNAs described in viruses, unicellular eukaryotes and a broad range of multicellular organisms, ranging from plants and insects to mammals. microRNAs control a variety of biological pathways including development, apoptosis, metabolism or immunological response by inhibiting protein translation and, in some cases, leading to degradation of mRNA transcripts. In view of numerous functions the connection of microRNAs with a wide range of human diseases comes as no surprise. Differences in microRNA expression patterns are significant in cancer, diabetes, heart malfunctions, neurodegenerative diseases *etc.* For this reason detection techniques for high-throughput microRNA profiling need to be developed. The novel small RNA labeling technology recruits the HEN1 methyltransferase to attach the extended side chains with functional group towards 3'-end of microRNA or siRNA. The method provides two strategies: quick and effortless one-step labeling through the direct attachment of the relevant reporter group which is embedded in the transferred radical. Alternatively, the two-step approach extends a choice of coupling strategies for manifold label conjugation to functional group.

202-B A short guide to human long non-coding RNA gene nomenclature*Matt Wright¹, Ruth Seal¹, Elspeth Bruford¹***¹HUGO Gene Nomenclature Committee (HGNC), EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK**

The HUGO Gene Nomenclature Committee (HGNC) is the only organisation authorised to assign standardised nomenclature to human genes. Of the 34,000 approved gene symbols in our database (www.genenames.org) the majority represent protein-coding (pc) genes; however we also name pseudogenes, phenotypic loci, some genomic features, and to date have named over 5,000 human non-protein coding RNA (ncRNA) genes and ncRNA pseudogenes. We have already established unique names for most of the small ncRNAs by working with experts for each class: for example we have named the ~1,500 human microRNA genes in collaboration with miRBase. Small ncRNAs can be defined into their respective classes by their shared homology and common function. In contrast long non-coding RNA (lncRNA) genes represent a disparate set of loci related only by their size, over 200 bases in length, share little conserved sequence homology, and have variable functions. As with pc genes wherever possible lncRNAs are named based on the known function of their product, e.g. '*XIST*' 'X (inactive)-specific transcript' is involved in transcriptionally silencing one of the pair of X chromosomes in females. We have currently named ~100 lncRNA genes that encode a transcript with published evidence of function. There are, however, potentially thousands of lncRNAs, and for the vast majority their function remains unresolved. Such lncRNA genes are named based on their genomic context. If there is a proximal pc gene then the lncRNA genes are given a gene symbol beginning with the pc symbol and assigned a suffix according to whether they are: antisense (AS) e.g. *BACE1-AS*; intronic (IT) e.g. *SPRY4-IT1*; or overlapping (OT) e.g. *SOX2-OT*. Whereas long intergenic lncRNAs (lincRNAs) that lie between pc gene loci are named with a common root symbol (*LINC*: "*long intergenic non-coding RNA*") and an iterated, numerical suffix. We present a short guide to the nomenclature of lncRNA genes and provide examples of some of the genes named to date. For further information on ncRNA nomenclature please see the HGNC RNA webpage: www.genenames.org/rna or email us at hgnc@genenames.org

717-B Maintenance of adult beta–cell identity by microRNAs and transcription factors

Amitai Mandelbaum, Sharon Kredo-Russo, Tal Melkman-Zehavi, Eran Hornstein

Hornstein lab, Department of Molecular Genetics, Weizmann Institute of Science

Normal physiology depends on defined functional output of differentiated cells. A century of Developmental–Biology studies revealed diverse mechanisms for differentiation, however once ‘terminally’ differentiated, cells are thought to naïvely stay put. However differentiated cells are surprisingly fragile, for example, phenotypic collapse and de–differentiation of beta–cells was recently discovered in pathogenesis of type 2 diabetes. These discoveries necessitate investigations of mechanisms for maintenance of robust cell–type identity over decades in the adult organism? microRNAs, which are small non-coding RNAs, are known to impart robustness to development. Our investigations reveal that microRNAs are providing means for continuous maintenance of adult beta–cell identity and therefore are important genetic components in metabolic disorders including in diabetes. This provides a new framework for miRNA function in adult tissues and in human disease.

Mechanisms of RNA interference

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 205 B – 220 B

Location: Main Hallway & Sanada Foyer

- 205 B** **Towards improved shRNA inhibitors with a Dicer-independent processing route**
- 208 B** **Endogenous RNA interference is driven by copy number**
- 211 B** **Single-molecule view on the action of Drosophila Dicer-2**
- 214 B** **Investigating the potential role of an archaeal Argonaute in the subtype III-B CRISPR system of *M. piezophila***
- 217 B** **P body-associated RNA silencing complex PRSC of *Cryptococcus* effects transposon suppression**
- 220 B** **Distinct activities of the *Dictyostelium discoideum* RNA-dependent RNA polymerases in post-transcriptional gene regulation**

205-B Towards improved shRNA inhibitors with a Dicer-independent processing route*Ben Berkhout¹*¹**University of Amsterdam**

Towards improved shRNA inhibitors with a Dicer-independent processing route Ben Berkhout Laboratory of Experimental Virology, Academic Medical Center, University of Amsterdam, The Netherlands (b.berkhout@amc.uva.nl) Short hairpin RNAs (shRNAs) are widely used to induce RNA interference (RNAi). The shRNA is processed by the Dicer endonuclease into an siRNA duplex. One strand of the duplex instructs the RNA-induced silencing complex (RISC) with the catalytic AGO2 protein to mediate cleavage of the complementary mRNA target. Although shRNAs can potently and specifically suppress target genes, RNAi may also cause serious side-effects, which is an siRNA-sequence and dose-dependent phenomenon. Thus, there is a need to select potent shRNAs in order to reduce the required shRNA concentration. To date, only few shRNA designs have been tested. We tested a variety of shRNAs that differed in stem length and terminal loop size and revealed strikingly different RNAi activities and shRNA processing patterns. Interestingly, we identified a specific shRNA design that uses an alternative Dicer-independent processing pathway, which also resulted in potent knockdown of the target gene. Detailed shRNA analyses indicated that a short stem length is critical for avoiding Dicer processing and activation of the alternative processing route, in which the shRNA is incorporated into RISC and processed by the AGO2-mediated slicer activity. Such alternatively processed shRNAs (AgoshRNAs) yield only a single RNA strand that effectively induces RNAi, whereas conventional shRNA processing results in an siRNA duplex of which both strands can trigger RNAi. These results have important implications for the future design of more specific RNAi therapeutics.

208-B Endogenous RNA interference is driven by copy number*Cristina Cruz¹, Jon Houseley¹*¹**The Babraham Institute**

A plethora of non-protein coding RNAs (ncRNA) are produced throughout eukaryotic genomes, many of which are transcribed antisense to protein-coding loci. A direct interaction between antisense ncRNA and protein-coding mRNA would form double-stranded RNA that could instigate an RNA interference (RNAi) response, resulting in mRNA down-regulation. However, this occurs very rarely and the variables controlling such an RNAi response remain undefined. Here we use a minimal reconstituted RNAi system in budding yeast to show that gene copy number is a key factor controlling the RNAi response to transcripts from endogenous loci, and that increasing copy number is sufficient to cause the degradation of both sense and antisense RNA by RNAi. This is observed for loci expressing ncRNA or mRNA, and occurs even with rare or unstable antisense transcripts. Importantly, increased RNA abundance does not account for this effect as multi-copy loci produce more small interfering RNAs (siRNA) than single-copy loci with equivalent RNA expression. The difference can instead be explained by the ability of multi-copy loci to simultaneously transcribe sense and antisense RNA. Cells are able to identify high-copy DNA, which is an essential step in the suppression of newly evolved transposable elements; our experiments clearly demonstrate that identification of the products of high-copy DNA is an emergent property of a minimal RNAi system. Efficient surveillance of high-copy sequences by RNAi would however require genome-wide transcription, suggesting a function for the pervasive transcription of eukaryotic genomes. We propose that pervasive transcription is part of a defence mechanism capable of directing a sequence-independent RNAi response against transposable elements amplifying within the genome.

211-B Single-molecule view on the action of *Drosophila* Dicer-2

Anna C. Haagsma, Mohamed Fareh, Chirlmin Joo

#N/A

RNA interference is a novel gene regulation process which involves small interfering RNAs (~20-30 nucleotides). The small interfering RNAs contribute to a multitude of cellular processes including development, metabolism and stress responses. The RNase III family enzyme Dicer is a specialized class of endonucleases that cleave double-stranded RNAs into small interfering RNAs [1, 2]. Despite the importance of the enzyme in RNA interference, the mechanism of Dicer is not yet fully understood. *Drosophila* has two types of Dicer with distinct biological functions. Among these two, Dicer-2 plays a critical role in transposon control and defense against viral infection. Dicer-2 processes a long double-stranded RNA into many short interfering RNAs. For efficient processing of a long double-stranded RNA, Dicer-2 may utilize a translocation mechanism through its helicase domain [3, 4]. By pulling down Dicer-2 out of insect cell extracts and immobilizing on a single-molecule imaging surface [5], we attempt to visualize the putative translocation process of the Dicer-2 protein for the first time.

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214-B Investigating the potential role of an archaeal Argonaute in the subtype III-B CRISPR system of *M. piezophila**Emine Kaya*¹, *Jennifer A. Doudna*²¹University of California, Berkeley; ²Department of Molecular and Cell Biology, Howard Hughes Medical Institute, Department of Chemistry, University of California, Berkeley, California 94720, USA and Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

Members of the Argonaute protein family are found in all higher eukaryotes and have important functions in translational regulation of gene products, as well as defense against viruses or transposable elements. In processes known as RNA interference (RNAi) and microRNA (miRNA) mediated silencing, Argonaute proteins associate with small non-coding RNAs and use them as guides to bind to complementary RNAs.(1) Instead of using RNAi as a defense mechanism against non-self genetic elements, archaeal and many bacterial genomes contain Clustered Regularly Interspaced Short Palindrome Repeats (CRISPR) and variable arrays of the CRISPR-associated (cas) genes, which together form the CRISPR-associated system (CASS).(2) In a manner analogous to RNAi, this adaptive immune system uses small RNAs to specifically target and degrade foreign genetic elements derived from phages and/or plasmids.

Despite the absence of homologous RNAi pathways, Argonaute proteins have been identified in prokaryotes. The crystal structures of several bacterial and archaeal Argonautes revealed the same domain architecture as their eukaryotic homologs. Furthermore, catalytically active prokaryotic Argonautes have been shown to bind small nucleic acid guides for endonucleolytic cleavage of complementary targets *in vitro*. However, the biological function of prokaryotic Argonaute proteins remains unknown.(3)

Recent phylogenetic analyses by *Makarova et al.* revealed the genomic colocalization of members of the Argonaute family and CASS in archaea.(4) Potentially active Argonaute proteins are encoded in the CRISPR subtype III-A and III-B loci in the archaeal species *Methanopyrus kandleri* and *Marinitoga piezophila*, respectively. In both genomes, the Argonaute protein is in the same operon as Cas1 and Cas2, which are likely to be responsible for spacer acquisition. Here, I present my recent efforts towards characterizing the CRISPR subtype III-B operon of *M. piezophila* and elucidating the role of Argonaute in this system.

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217-B P body-associated RNA silencing complex PRSC of *Cryptococcus* effects transposon suppression

*Prashanthi Natarajan*¹, *Joshua Dunn*¹, *Jonathan Weissman*¹, *Phillip Dumesic*¹, *Hiten Madhani*¹

¹University of California San Francisco

We recently described a novel RNA silencing system in the yeast *Cryptococcus neoformans* (Dumesic, Natarajan et al., *Cell*, 152, 957-968, 2013), in which stalled spliceosomes serve as a signal for RNAi-mediated genome defense. In addition to the nuclear SCANR complex required for siRNA accumulation, we described PRSC, a P body-associated RNA silencing complex. PRSC is dispensable for siRNA accumulation, suggesting it is an effector complex. PRSC contains two subunits: the Argonaute, Ago1 and a glycine-tryptophan(GW) motif protein, Gwo1. Gwo1 and mammalian GW182 family members are best reciprocal protein search hits suggesting they are orthologs. Together with their similar association with Argonaute proteins and their localization to P-bodies, the effector complexes from the yeast and mammalian systems appear to be striking similar. Consistent with this view, PRSC physically associates with mRNAs targeted by small RNAs in a siRNA-dependent manner. To probe the biological function of RNA silencing, we developed a quantitative *in vivo* transposon mobilization assay using a marked *Harbinger* DNA transposon. We found PRSC and SCANR are both required for suppression of transposon mobilization *in vivo*: specifically, we observed a 1000-fold increase in the frequency excision events in null mutants in either complex, supporting the view that PRSC is a genome defense effector complex. Despite the magnitude of its effect on transposon mobilization, cells lacking PRSC display only modest increases in target RNA levels. This raised the possibility that that PRSC, like mammalian GW182-Argonaute complexes, controls the translation of targets mRNAs. To probe the mechanistic role of PRSC, we successfully implemented ribosome profiling in *Cryptococcus* to examine the impact of PRSC on transcriptome-wide ribosome occupancy. Insights into the role of PRSC obtained from these experiments will be described.

220-B Distinct activities of the *Dictyostelium discoideum* RNA-dependent RNA polymerases in post-transcriptional gene regulation

Stephan Wiegand¹, Carsten Seehafer¹, Marek Malicki¹, Patrick Hofmann⁵, Annika Schmith², Thomas Winckler², Balint Földesi⁶, Benjamin Boesler⁶, Wolfgang Nellen⁶, Johan Reimegård⁴, Lotta Avesson³, Fredrik Söderbom⁷, Christian Hammann¹

¹Jacobs University Bremen; ²Friedrich-Schiller-Universität Jena; ³Garvan Institute of Medical Research; ⁴KTH Royal Institute of Technology; ⁵TU Darmstadt; ⁶University of Kassel; ⁷Uppsala University

Cellular RNA dependent RNA polymerases (RdRPs) are involved in various processes of gene regulation and different mechanisms for their action have been proposed. To study the function of the three RdRPs in the amoeba *Dictyostelium discoideum*, we have deleted the encoding genes *rrpA*, *rrpB* and *rrpC* in all possible combinations. We show that the two RdRPs RrpA and RrpC of the amoeba exert specific, non-overlapping roles in the regulation of retrotransposons and microRNAs.

Strains lacking RrpC strongly accumulate transcripts of the centromeric retrotransposon DIRS-1 and show a dramatic loss of DIRS-1 small RNAs, which are asymmetrically distributed in the wild type, as shown by deep sequencing. We report the discovery of an hitherto unknown long antisense DIRS-1 transcript that is driven by the promoter activity of the right inverted long terminal repeat. Fluorescence *in situ* hybridization shows both this long antisense transcripts and the sense RNA in nuclear spots. We propose that in wild type cells, both DIRS-1 transcripts are generated, but post-transcriptionally degraded by the action of RrpC, possibly with the help a Dicer-related nucleases in the amoeba. By this, RrpC apparently serves to prevent retrotransposition, as we observe a strong accumulation of DIRS-1 copies in the genome of the *rrpC* gene deletion strain.

In contrast to this, the silencing of another retrotransposon, Skipper, appears to be mediated by RrpA and the Dicer-related nuclease DrnB. Deletion strains of the respective genes display strongly increased Skipper transcript levels, but here, surprisingly, also small RNA levels are elevated. Such increased RNA levels in strains lacking RdRPs were also observed for other types of small RNAs, including microRNAs.

To study the activity of the RdRPs molecularly, we have investigated in the *rrp* gene deletion strains the appearance of small RNAs derived by antisense or hairpin RNA constructs against the transgene *lacZ*. Both types of constructs led to a similar reduction of the enzymatic activity of β -Galactosidase. However, only in *rrpC* knock out strains, low levels of β -gal small interfering RNAs (siRNAs) could be detected in antisense RNA expressing strains. In contrast to this, and at considerably higher levels, all hairpin RNA expressing strains featured β -gal siRNAs. Spreading of the silencing signal to mRNA sequences 5' of the original hairpin trigger was observed in all strains, except when the *rrpC* gene or that for the Dicer-related nuclease DrnB was deleted, indicating that transitivity of an RNA silencing signal exists in *D. discoideum* and that it requires the two enzymes RrpC and DrnB.

In summary, our data indicates that the RdRPs RrpA and RrpC have specific endogenous targets in *D. discoideum* and that gene silencing triggered by antisense RNA and hairpin RNA works through different mechanisms despite an overall similar efficiency.

RNA and Epigenetics

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 223 B – 229 B

Location: Main Hallway & Sanada Foyer

- 223 B** **Characterization of small RNAs that contribute to ribosomal RNA gene silencing through the siRNA machinery**
- 226 B** **Human Argonaute Suppresses Cryptic RNA Polymerase II Transcription and Chromatin Structure of the Silent rRNA Genes.**
- 229 B** **Single cell analysis reveals aspects of antisense RNA regulation and mode of action in PHO84 transcription repression**

223-B Characterization of small RNAs that contribute to ribosomal RNA gene silencing through the siRNA machinery*Blake Atwood¹, Keith Giles¹***¹University of Alabama at Birmingham**

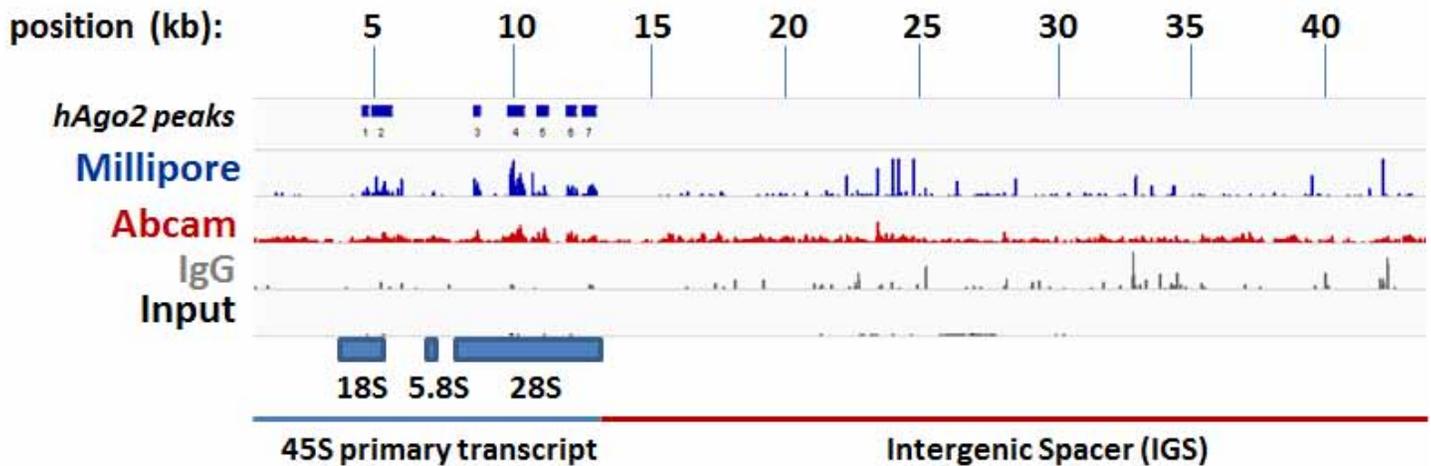
Cellular growth and division is limited by the number of ribosomes actively translating mRNA in the cell. The ribosomal RNA gene is highly transcribed making up to 80% of total RNA transcribed in the cells with the deregulation thereof leading to abnormal cellular growth and cancer. In normal human haploid cells there are approximately 200 copies of the ribosomal RNA gene of which half are maintained in a silent and heterochromatic state. Maintaining the ratio of active to silent ribosomal DNA loci seems to be critical in maintaining normal cellular homeostasis. The major Polymerase that transcribes the ribosomal DNA locus is RNA Pol I. Data suggest that Pol II is also localized to the ribosomal DNA locus and that the low level of transcription by Pol II aids in maintaining the silent loci in a heterochromatic state. These Pol II transcripts of the ribosomal DNA locus provide Argonaute 2 with small RNAs which allow it to be in turn targeted to the ribosomal DNA. Argonaute 2 then recruits the necessary silencing machinery to maintain the silent loci in their heterochromatic state thus maintaining a normal cellular growth rate. The major goal of this work is to characterize the small RNAs transcribed by Pol II and how they feed into the ribosomal DNA silencing pathway.

226-B Human Argonaute Suppresses Cryptic RNA Polymerase II Transcription and Chromatin Structure of the Silent rRNA Genes.

Keith Giles¹, Gaelle Lefevre², Blake Atwood¹, Mariana Saint Just Ribeiro¹, Gary Felsenfeld²

¹University of Alabama at Birmingham; ²NIDDK/NIH

Eukaryotic cells package much of their genome into a heterochromatic structure in order to facilitate the proper regulation of gene expression and nuclear organization. In *S. pombe*, the initiation of heterochromatin structure is mediated by the highly conserved RNAi machinery but it remains unclear if RNAi performs a similar function in humans. To examine this question, we performed a genome-wide analysis of the chromatin binding sites of hAgo2 in the human erythroleukemia cell line, K562. Our results suggest that hAgo2 is found almost exclusively within repetitive DNA sequences, with a strong preference for the coding region of the silent ribosomal DNA loci (Figure 1, below). This localization is Dicer-dependent, and correlates with Dicer-processed, hAgo2-bound, small RNAs. Knockdown of hAgo2 causes a ~10% increase in the synthesis rate of the mature 28S and 18S rRNAs. However, a knockdown of hAgo2 causes a ~3-fold increase in synthesis of cryptic RNA species originating from the rRNA gene body. This increase corresponds with a 3-fold increase in Pol-II localization. Ago2 is needed to maintain the proper histone modification pattern, as a loss of hAgo2 causes a loss of H3K9me2 and an increase in H4ac throughout the locus. This change in histone modification pattern is likely a consequence of the loss of Suv39H1 recruitment in hAgo2 KD cells. Consistent with a role in regulating only the silent rRNA genes, hAgo2 does not localize within the nucleolus and has no effect on the recruitment of the RNA Polymerase I transcription factor, UBF1. This interaction is cell cycle specific; hAgo2 is lost from the rRNA genes during M-phase. We present our model of cooperation between hAgo2 and Pol-II in maintaining the proper chromatin structure among the silent rRNA genes.



229-B Single cell analysis reveals aspects of antisense RNA regulation and mode of action in *PHO84* transcription repression

*Samir Rahman*¹, *Manuele Castelnuovo*², *Elisa Guffanti*², *Francoise Stutz*², *Daniel Zenklusen*¹

¹Université de Montréal; ²University of Geneva

A large number of non-coding RNAs (ncRNAs) are transcribed in yeast; many of them are rapidly degraded by the nuclear exosome. The function of most of these ncRNAs, however, is still largely unknown, although a subset has been implicated in modulating transcription regulation. Loss of nuclear exosome component Rrp6 results in the accumulation of long *PHO84* antisense RNAs and repression of sense transcription in a mechanism that involves *PHO84* promoter deacetylation by the Hda1/2/3 histone deacetylase complex. Moreover, the Set1 H3K4 methyl transferase stimulates antisense RNA production, correlating with *PHO84* repression, the precise mechanisms that orchestrates *PHO84* transcriptional silencing however is still not fully understood. Here, we use single molecule resolution fluorescent in situ hybridization (smFISH) to further investigate ncRNA mediated transcription regulation of *PHO84* at the single cell level. We show that *PHO84 AS* transcription acts as a bimodal switch in *PHO84* regulation, where low frequency *PHO84 AS* transcription efficiently represses sense transcription within individual cells. Surprisingly, *PHO84 AS* RNAs do not accumulate at the *PHO84* gene but are exported to the cytoplasm where they are degraded by the 5'-3' RNA degradation machinery. Furthermore, we show that loss of Rrp6 promotes antisense production by reducing early transcription termination by the Nrd1/Nab3/Sen1 complex, rather than by stabilizing the *PHO84 AS* RNA, and that loss of Set1 enhances early termination by promoting Nrd1 recruitment. These observations suggest that antisense-mediated repression is regulated, at least in part, at the level of antisense early termination, and that *PHO84* silencing results from low frequency yet constant antisense transcription through the promoter rather than its static accumulation at the repressed gene.

Small RNAs

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 232 B – 256 B

Location: Main Hallway & Sanada Foyer

- 232 B** Introns of plant pri-miRNAs are required for proper biogenesis and function of miRNAs
- 235 B** miRNA profiles characterise distinct states of cellular pluripotency
- 238 B** Positive regulation of inflammation by miR-19
- 241 B** Temporal expression of tRNA fragments in development of *Triops cancriformis* (Tadpole shrimp)
- 244 B** A Specialized Mechanism of Translation Regulation in Quiescence
- 247 B** Competition between spliceosome and microprocessor complex regulates processing of Splice site Overlapping (SO) 34b pri-miRNA
- 250 B** Poly(A) and histone mRNA processing factor Symplekin is involved in endo-siRNA biogenesis
- 253 B** The role of the RNA chaperone protein Hfq in the translation regulation by small noncoding RNAs targeting ompD mRNA from *Salmonella typhimurium*.
- 256 B** An Exchange-Induced Remnant Magnetization Technique for MicroRNA Detection

232-B Introns of plant pri-miRNAs are required for proper biogenesis and function of miRNAs

*Dawid Bielewicz*¹, *Malgorzata Kalak*¹, *Maria Kalyna*³, *David Windels*², *Andrea Barta*³, *Franck Vazquez*², *Zofia Szweykowska-Kulinska*¹, *Artur Jarmolowski*¹

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Plant *MIR* genes are independent transcription units that encode long primary miRNA precursors which usually contain introns. For two miRNA genes, *MIR163* and *MIR161*, we show that introns are crucial for the accumulation of proper levels of mature miRNA. Removal of the intron in both cases led to a drop-off in the level of mature miRNAs. We demonstrate that the stimulating effects of the intron mostly reside in the 5'ss rather than on a genuine splicing event. Our findings are biologically significant since the presence of functional splice sites in the *MIR163* gene appears mandatory for pathogen-triggered accumulation of miR163 and proper regulation of at least one of its targets.

235-B miRNA profiles characterise distinct states of cellular pluripotency

*Jennifer Clancy*¹, *Hardip Patel*¹, *Nicole Cloonan*², *Andrew Corso*³, *Mira Puri*³, *Pete Tonge*³, *Andras Nagy*³, *Thomas Preiss*¹

¹John Curtin School of Medical Research, Australian National University, Australia; ²Genomic Biology Laboratory, Queensland Institute of Medical research, Australia; ³Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada

Reprogramming of fibroblasts through induction of the Yamanaka factors (Myc, Sox2, Klf4 and Oct4) is a powerful approach to delineate the molecular characteristics of the pluripotent cellular state. We used an efficient secondary reprogramming system to monitor whole culture reprogramming over time, allowing us to characterise intermediate states as well as to identify several distinct pluripotent cell states. As part of this investigation we measured the miRNA profiles of these cell states by next-generation sequencing, which feature marked changes in the expression of many of the previously described core miRNA-mediators of pluripotency. We uncover the timing of their involvement in the process of pluripotent cell generation, which sheds light on their individual roles in the process, as well as on how miRNAs are regulated during reprogramming. We describe the miRNA processing variants that exist in these cell states, including 5' and 3' isomiRs, non-templated addition, editing and unusual strand bias. Many of the core reprogramming miRNAs have isomiRs, which can alter their targeting spectrum and lead to reinterpretation of their specific roles. Broader analysis of the dataset also suggests that other small RNA species may be involved in pluripotency as, like the miRNA population itself, the small RNA profile changes markedly after induction of the Yamanaka factors. This work is part of a larger collaborative study, which co-ordinately measured protein, miRNA, mRNA, DNA methylation and histone modification in this model of pluripotent cell generation and related data will be discussed in the context of miRNA regulation and function.

238-B Positive regulation of inflammation by miR-19*Michael Gantier¹, H. James Stunden², Bryan Williams¹***¹Monash Institute of Medical Research, Monash University, Clayton, Victoria, Australia; ²Institute of Innate Immunity, University Hospitals, University of Bonn, Bonn, Germany**

microRNAs (miRNAs) are critical for normal cell function, and several miRNAs (e.g. miR-146a) have already been found to be involved in the repression of inflammation. To identify miRNAs that enhance inflammation, we performed *in silico* bioinformatic analyses of several known negative regulators of NFκB activity. Putative targeting by miRNAs from the oncogenic miR-17~92 cluster was enriched in such genes. Selective inhibition of each member of the miR-17~92 cluster demonstrated an important role for miR-19b in the positive regulation of NFκB signaling. We confirmed direct targeting of several key negative regulators of NFκB signaling by miR-19b, including A20 and its partner RNF11. Specific depletion of miR-17~92 in primary mouse macrophages of miR-17~92 conditional knock out mice resulted in dampened production of pro-inflammatory cytokines following innate immune activation. In addition, transfection of rheumatoid arthritis primary synovial fibroblasts with miR-19b mimics resulted in increased IL-8 production by these cells, establishing a pro-inflammatory effect of miR-19b in the context of disease. Our results thereby establish the coordinate regulation of a network of regulators of NFκB activity by miR-19, promoting inflammation.

241-B Temporal expression of tRNA fragments in development of *Triops cancriformis* (Tadpole shrimp)

*Yuka Hirose*¹, *Kahori Ikeda*¹, *Emiko Noro*¹, *Kiriko Hiraoka*¹, *Masaru Tomita*¹, *Akio Kanai*¹

¹Institute for Advanced Biosciences, Keio University

It is well known that microRNAs, which are 18-24 nucleotide (nt) regulatory RNAs, are deeply implicated in development, especially in morphogenesis. However, the relationship between other sizes of small RNAs (sRNAs) and development remains obscure. In this research, we chose *Triops cancriformis* (Tadpole shrimp) for target organisms since its morphology changes dramatically during the development, and focused on stage-specific sRNAs range in sizes from 25 to 45 nt.

In order to find the stage-specific sRNAs, we performed deep sequencing analysis of sRNA libraries constructed from each six developmental stage (egg, 1st-4th instar larvae, and adult) of *T. cancriformis*. After removing unreliable sequencing reads, novel sRNA candidates of 25-45 nt long were extracted. Based on the analysis of their gene expression (read counts) as well as comparative genomics between the candidate sRNAs and known non-coding RNAs in other species, many putative tRNA fragments were detected in any one of six developmental stages. To reveal the exact origin of these tRNA fragments, it is necessary to know the mature tRNA sequences. Therefore, we also conducted deep sequencing analysis of genomic DNA isolated from *T. cancriformis* and predicted a set of tRNA genes using tRNAscan-SE. As a result, at least five tRNA fragments were found to be derived from either tRNA^{Gly}(CCC), tRNA^{Lys}(CUU), tRNA^{Glu}(CUC), tRNA^{Asp}(GUC), or tRNA^{Phe}(GAA). Moreover, northern blot analysis showed that these tRNA fragments were actually expressed in *T. cancriformis*. Interestingly, in the case of the tRNA^{Lys}(CUU), several tRNA fragments in different length were produced depending on the developmental stages. These results suggest that tRNA fragments are not random degradation products, but may have some important role(s) in eukaryotic development. Recently, it has been demonstrated that tRNA fragments are generated by endonucleolytic cleavage of tRNAs under specific conditions such as age, cancer, oxidative stress and amino-acid-starvation in various cell types. The possible function(s) of tRNA fragments in the development of *T. cancriformis* are discussed in the conference.

244-B A Specialized Mechanism of Translation Regulation in Quiescence*Sooncheol Lee¹, Samuel Truesdell¹, Syed Irfan Bukhari¹, Ju Huck Lee¹, Olivier Le Tonqueze¹, Shobha Vasudevan¹*¹**Center for Cancer Research, Massachusetts General Hospital, Harvard Medical School, Boston, USA**

Quiescence (G0) represents an assortment of reversible, proliferation-arrested states, implicated in the persistence of clinically resistant cancer cells, dormant stem cells and other distinct, arrested cells in development and in the body. The G0 state involves a discrete gene expression program with selective mRNA expression while decreasing general translation. One subset of such transcripts includes select mRNAs recruited by a distinct microRNP (microRNA-protein complex) containing Argonaute 2 (AGO2) and a specific isoform of Fragile X-mental-retardation-related protein 1, isoform-a (FXR1a). The AGO-FXR1a microRNP lacks the repressor, GW182, and promotes translation of associated mRNAs in G0 mammalian cells and in G0-like immature *Xenopus laevis* oocytes. Our data reveal that microRNA-mediated upregulation is dependent on nuclear entry of the microRNA in immature oocytes; cytoplasmic injections result in repression. FXR1a overexpression rescues translation upregulation of cytoplasmically-injected RNAs and in low density, proliferating cells. Consistently, *in vivo* crosslinking-coupled nuclear-cytoplasmic fractionation and immunoprecipitation demonstrate significant interaction of AGO with FXR1a in the nucleus compared to the cytoplasm. MicroRNA targets for upregulation, Myt1 and TNFa mRNAs and reporters bearing their target sequences, are associated with the nuclear AGO-FXR1a microRNP. mRNAs that are repressed or lack target sites are not associated with this nuclear microRNP, indicating the importance of a compartmentalized AGO-FXR1a microRNP for selective mRNA recruitment for translation upregulation. The AGO-FXR1a microRNP interacts with a specialized translation factor in G0 and in immature oocytes, thereby connecting mRNAs recruited by this microRNP with the translation machinery for selective expression. Importantly, disruption of this mechanism in G0 abrogates microRNA-mediated translation upregulation and decreases G0 cell viability, indicating its relevance for maintenance of the quiescent state.

247-B Competition between spliceosome and microprocessor complex regulates processing of Splice site Overlapping (SO) 34b pri-miRNA*Franco Pagani¹, Chiara Mattioli¹, Giulia Pianigiani¹***¹Human Molecular Genetics ICGEB. Trieste. Italy**

Several coding or non-coding PolII precursor transcripts are processed into both spliced mRNAs and micro RNAs (miRNA) but the mechanism that coordinates the spliceosome and the Drosha-DGCR8 Microprocessor complex (MPC) activities are not completely understood. We have explored the functional relationships between these two machineries in a peculiar class of miRNAs, we named Splice site Overlapping (SO)-miRNA, whose pri miRNA hairpins overlap with splice sites. We found 17 pri-miRNA hairpins overlapping with splice sites, 11 SO pri-miRNAs contain a 3'ss, 6 a 5'ss and 8 are evolutionarily conserved among vertebrates. We focussed on the evolutionarily conserved SO miR-34b whose non-canonical 3'ss lacks a polypyrimidine tract. miR34, originally identified as a tumour suppressor miRNA, is involved in several physio-pathological conditions including spermatogenesis, neurodegeneration, central stress response and neural stem cell differentiation.

SO pri-miR-34b hairpin is located in the last exon of a non-coding transcript and we show that the embedded non-canonical acceptor site is correctly spliced *in vivo* in human tissues and in minigene systems. Through mutational analysis we identify two indispensable elements for the recognition of the non-canonical site: a strong branch point located in the hairpin, 18 bp upstream of the 3'ss AG dinucleotide, and a downstream purine-rich exonic splicing enhancer (ESE). Interestingly, in minigene systems, splicing inhibition due to ESE deletion or direct disruption of the AG 3'ss increases miR-34b levels. On the other hand, siRNA-mediated silencing of Drosha and/or DGCR8 improves splicing efficiency and abolishes miR-34b production. Thus, the processing of this 3' SO miRNA is regulated in an antagonistic manner by the MPC and the spliceosome. We propose that in SO miRNAs, competition between these two machineries on the nascent transcript represents a novel mechanism to regulate miRNA biosynthesis.

250-B Poly(A) and histone mRNA processing factor Symplekin is involved in endo-siRNA biogenesis*Mindy Steiniger*¹, *William Marzluff*²¹University of Missouri-St. Louis; ²University of North Carolina-Chapel Hill

CPSF73, CPSF100 and Symplekin form a protein complex required for 3' end processing of both histone mRNAs and canonical polyadenylated mRNAs. CPSF73 is the cleavage factor, CPSF100 forms a heterodimer with CPSF73 and Symplekin is a scaffolding protein that binds CPSF73, CPSF100 and several other proteins. This "core cleavage complex" interacts with various accessory factors to cleave mRNAs with different 3' ends. In an attempt to isolate other proteins involved in histone 3' end processing, we performed a large-scale immunoprecipitation of Symplekin from nuclear extracts of *Drosophila* tissue culture cells. A protein specific antibody was used to isolate endogenous Symplekin, samples were separated on an SDS-PAGE gel and mass spectrometry was performed to identify bound proteins. Several known Symplekin-interacting proteins, including CPSF160, CPSF100, CPSF73 and CstF77, were isolated in addition to many factors previously not reported to be involved in polyadenylation. I also found an unexpected binding partner: Dicer-2 (Dcr-2). Dcr-2 processes dsRNAs into both endo- and exo-siRNAs in *Drosophila melanogaster*. To confirm the Symplekin-Dcr-2 interaction, I did the reciprocal IP with an anti-Dcr-2 antibody. This experiment confirms the Dcr-2-Symplekin interaction. To determine if Symplekin is necessary for the production of endogenous siRNAs, I RNAi-depleted Dmel-2 tissue culture cells of Symplekin and performed a northern blot with a probe to esi-2.1, an endogenous siRNA. This experiment shows that when Symplekin is RNAi-depleted, the amount of esi-2.1 produced is drastically reduced. Preliminary data indicate that Symplekin may be required for small RNA biogenesis, in addition to its role in 3' end processing.

253-B The role of the RNA chaperone protein Hfq in the translation regulation by small noncoding RNAs targeting *ompD* mRNA from *Salmonella typhimurium*.

*Zuzanna Urbaniak*¹, *Aleksandra Kaszynska*¹, *Joanna Stróżecka*¹, *Daria Grygiel*¹, *Mikolaj Olejniczak*¹

¹**Department of Biochemistry, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University**

The RNA chaperone protein Hfq is a hexameric Sm-like protein, which acts as a major-regulator of bacterial gene expression. Hfq facilitates the binding of sRNAs to their target mRNAs and affects the stability of their complexes. It has been proposed that Hfq is recruited to the regulated mRNAs through (ARN)_n motifs, and that the mRNA-sRNA annealing is most accelerated when Hfq binds mRNA immediately 3' of the sRNA binding site. Interestingly, the mRNA of *Salmonella* outer membrane protein OmpD is regulated by four Hfq-dependent sRNAs (InvR, MicC, RybB, SdsR), which bind partly overlapping sites located downstream of the AUG codon. The region of *ompD* mRNA containing the sRNA binding sites also includes several ARN repeat sequences, which could serve as potential Hfq binding sites.

The aim of this project is to elucidate the role of Hfq for the binding of different sRNAs to the *ompD* mRNA sequence. To characterize the structures of interacting molecules the *in vitro* structure probing of 5'-end-labeled RNAs in the absence or presence of Hfq was performed. The comparison of cleavage patterns allowed us to determine sRNA secondary structures and their Hfq binding sites. As the mRNA binding sequences of SdsR and InvR sRNAs are located in base-paired regions it appears possible that the Hfq role in facilitating the sRNA-mRNA annealing could be related to unfolding these regions. Next, we are planning to analyze the role of potential Hfq binding sites on *ompD* mRNA for promoting the sRNA-mRNA annealing. The results of these experiments could allow us to better understand the role of the *Salmonella* Hfq protein in facilitating sRNA interactions with their target mRNAs.

This study was supported by grants from the National Science Center (nr 2011/01/B/NZ1/05325) and the Foundation for Polish Science (TEAM/2011-8/5).

256-B An Exchange-Induced Remnant Magnetization Technique for MicroRNA Detection*Shoujun Xu¹, Li Yao¹, Yuhong Wang²*¹Department of Chemistry, University of Houston; ²Department of Biology and Biochemistry, University of Houston

We present a novel technique of exchange-induced remnant magnetization (EXIRM) for label-free microRNA (miRNA) detection. The characteristics of this technique are the following: ultrahigh sensitivity, broad dynamic range, single-base specificity, and no use of amplification or washing. MiRNAs are important regulators of genes and biomarkers for cancer, but their detection and sequencing remain challenging for existing techniques. The short length of miRNAs, which usually contains 18-25 nucleotides, makes precise sequencing difficult using conventional techniques. The expression levels of miRNAs are highly heterogeneous, which requires high sensitivity and broad dynamic range for the detecting methods.

The EXIRM technique is based on a novel concept that can be described in the following three stages. First, RNA duplexes are prepared on the sample well surface. One of the two strands is complimentary to the target miRNA and immobilized on the surface; the other strand has one mismatching base and is labeled with magnetic particles. Second, the sample containing various target miRNAs are incubated in the sample well with the initial RNA duplex. Exchange reaction takes place because the target miRNA has stronger binding with its complimentary RNA than the mismatching strand has. Third, the dissociated mismatching strand undergoes Brownian motion, causing randomization of the magnetic dipoles of the magnetic particles. Therefore, the quantity of the target miRNA is measured as a magnetic signal decrease. The detection of magnetic signals is achieved by an atomic magnetometer, the most sensitive device for magnetic sensing. The dynamic range of detection is at least five orders of magnitude and can be expanded by varying the size of the magnetic labels. The unique combination of zeptomole sensitivity, single-base specificity, and broad dynamic range makes EXIRM a well suited technique for miRNA identification.

RNA Catalysis and Riboswitches

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 259 B – 271 B

Location: Main Hallway & Sanada Foyer

- 259 B** **Molecular Mechanism of preQ1 Riboswitch Action: a Molecular Dynamics Study**
- 262 B** **Studying Conformational Changes in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy**
- 265 B** **THEORETICAL METHODS SUGGEST THAT SMALL RIBOZYMES MAY USE MULTICHANNEL MECHANISM DURING CATALYSIS**
- 268 B** **Metal Ion Requirement and Substrate Specificity of Kinase Ribozyme K28(1-77)C**
- 271 B** **The catalytic mechanism of a novel, widely disseminated small nucleolytic ribozyme**

259-B Molecular Mechanism of preQ1 Riboswitch Action: a Molecular Dynamics Study

Pavel Banáš¹, Petr Sklenovský¹, Joseph E. Wedekind², Jiri Šponer³, Michal Otyepka¹

¹Department Physical Chemistry and RCPTM, Palacky University Olomouc, Czech Republic.; ²Department Of Biochemistry and Biophysics, University of Rochester, Rochester, USA(NY); ³Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic; CEITEC, Masaryk University, Brno, Czech Republic

Riboswitches often occur in the 5'-untranslated regions of bacterial mRNA where they regulate gene expression. The preQ₁ riboswitch controls the biosynthesis of a hypermodified nucleoside queuosine in response to binding the queuosine metabolic intermediate. Structures of the ligand-bound and ligand-free states of preQ₁ riboswitch from *Thermoanaerobacter tengcongensis* were determined recently by crystallography.¹ We used multiple, microsecond-long molecular dynamics simulations (29 μs in total) to characterize the structural dynamics of preQ₁ riboswitch in both states. We observed different stability of the stem bearing part of the Shine-Dalgarno sequence in the bound and free states, resulting in different accessibility of the this ribosome-binding site. These differences are related to different stacking interaction between nucleotides of the stem and the adjacent RNA loop, which itself adopts different conformations in the bound and free states.^{1,2} We suggest that the loop serves not only to bind preQ₁ but also transmits information about ligand binding from the ligand-binding pocket to the stem, which has implications for mRNA accessibility to the ribosome.² We explain functional results obscured by a high salt crystallization medium and help to refine regions of disordered electron density, which demonstrates the predictive power of our approach.²

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262-B Studying Conformational Changes in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy*Jackie Esquiaqui¹, Gail Fanucci¹***¹University of Florida**

Electron Paramagnetic Resonance (EPR) spectroscopy is used to study dynamic conformational changes in the RNA glycine riboswitch. The dynamic role of the leader-linker interaction within glycine riboswitch conserved sequences is probed through site directed spin labeling and continuous wave EPR. Inter-aptamer and aptamer-expression platform interactions are elucidated through double electron-electron resonance spectroscopy. Incorporation of spin labels is achieved through optimized ligation methodologies allowing synthetically modified RNA to be joined to larger RNA sequences. Expected folding and burial of riboswitch elements will lead to restricted motion of the spin label and, additionally, pulsed EPR experiments yield distance distribution profiles indicating conformational exchange between states in the absence and presence of glycine.

265-B Theoretical methods suggest that small ribozymes may use multichannel mechanism during catalysis*Vojtech Mlynsky¹, Pavel Banas¹, Nils G. Walter², Jiri Sponer³, Michal Otyepka¹*¹Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Palacky University Olomouc, Czech Republic; ²Department of Chemistry, Single Molecule Analysis Group, University of Michigan, Ann Arbor, MI, USA; ³Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Small self-cleaving ribozymes carry out site-specific cleavage and ligation of RNA substrates.[1] Experiments and biochemical studies identified nucleobases and hydrated magnesium ions as catalytically active species, but their exact roles in reaction mechanisms remain questionable.[2,3] We studied extensively two members of the group of self-cleavage ribozymes: hepatitis delta virus (HDV) and hairpin ribozymes.

X-ray structures of ribozymes have to be mutated or chemically modified in order to trap them in precursor-like states. We carried out all-atom molecular dynamics (MD) simulations in explicit solvent up to μ s-time scales, which helped us to obtain native arrangement of the catalytically active sites. We tested the impact of different protonation states of key nucleobases on structural stability and compactness of active sites within both ribozymes. Finally, we used these MD simulations to generate geometries of the ribozymes in potentially reactive forms. These structures were further analyzed by the hybrid quantum-mechanical/molecular mechanical (QM/MM) calculations to decipher exact roles of chemical species (such as (de)protonated nucleobases and/or hydrated magnesium ion) involved in the catalysis. We used hybrid DFT functional MPW1K for the QM region, which was carefully validated against chemically accurate CCSD(T)/CBS method.[4] We found that the MPW1K error in energies is less than 1 kcal/mol. The observed potential energy surfaces (PESs) of the sugar-phosphate backbone self-cleave reactions in both ribozymes are rather complex, therefore the transition states were localized using two-dimensional scans of PES.[5,6] In both ribozymes, we identified several reaction mechanisms having comparable free energy barriers (and thus comparable rate constants), which allowed us to suggest a hypothesis about multichannel mechanism of the sugar-phosphate backbone self-cleavage in these ribozymes.

Acknowledgements

This work was supported by the Operational Program Research and Development for Innovations - European Regional Development Fund (CZ.1.05/2.1.00/03.0058) and European Social Funds (CZ.1.07/2.3.00/20.0058, CZ.1.07/2.3.00/20.0017). Grant Agency of the Czech Republic (P208/12/G016, P208/12/1878) and NIH grant 2R01 GM062357 are also gratefully acknowledged.

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268-B Metal Ion Requirement and Substrate Specificity of Kinase Ribozyme K28(1-77)C*Raghav Poudyal*¹, *Elisa Biondi*², *Josh Forgy*³, *Phuong Nguyen*⁴, *Donald Burke*⁵¹University of Missouri-Department of Biochemistry; ²Foundation for Applied Molecular Evolution-720 SW 2nd Ave, Suite 207, Gainesville, FL - 32601; ³University of Missouri; ⁴University of Missouri; ⁵University of Missouri-Department of Molecular Microbiology and Immunology, Department of Biochemistry, Bond Life Sciences Center

The RNA world hypothesis posits that Ribonucleic Acids (RNA) played a major role as the repository for genetic material and as catalysts during the early evolution of life and metabolism. Phosphoryl transfer is one of the key reactions in metabolic pathways, signal transduction and gene regulation in all domains of contemporary life. In vitro selection of functional nucleic acids has identified multiple kinase ribozymes but much remains to be known about the mechanisms utilized by these ribozymes to carry catalysis. It is therefore important to understand the strategies used by kinase ribozymes to perform metabolically relevant reactions. Our lab selected ribozyme K28(1-77)C, a dual-site self(thio)phosphorylating kinase ribozyme that utilizes Cu²⁺ for catalysis. We used in-line cleavage assay to assess the role of Cu²⁺ during the self(thio)phosphorylation. Modulation of in-line cleavage was observed only when GTP(phosphoryl donor) or both GTP and Cu²⁺ were present while we saw no evidence of direct Cu²⁺-ribozyme interaction. Addition of competitor for Cu²⁺ resulted in the loss of in-line cleavage signal which is restored by supplementation with Cu²⁺. Self (thio) phosphorylation assays with competitors also showed similar results; loss of activity with competitor for Cu²⁺ and restoration with Cu²⁺ supplementation. These experiments suggest that Cu²⁺-GTP complex is recognized by the ribozyme. Although 6 other families of ribozymes were isolated from the same selection, we found that only K28 (1-77)C is dependent upon Cu²⁺ for phosphoryl transfer catalysis.

To understand the substrate specificity for phosphoryl acceptor, we have assembled the ribozyme in a two-strand construct; a 22-nt substrate and a 44-nt catalytic strand. All kinase ribozymes studied to date have used the 2'OH or the 5'OH of ribose sugars as phosphoryl acceptors; however, we show here that the 5' substrate strand of the ribozyme can be made entirely of DNA. The DNA substrate gets phosphorylated even when the 5' end is blocked with biotin. We are proposing that the catalytic strand of the two stranded version of ribozyme K28 (1-77) C is the first kinase ribozyme that directly phosphorylates a nucleobase of its nucleic acid substrate. Atomic level mutations on a critical 5' triplet GGA suggest that 2-amino moiety in the two Guanosines plays important role during catalysis plausibly as hydrogen bond donors. We are currently studying the pH dependence of the reaction to identify potential role of Electron Donating Groups (EDGs) to activate the nucleophile for the reaction. Phosphoryl transfer on the nucleobase is an important step during the de novo biosynthesis of nucleotides such as ATP. Study of this ribozyme could help us understand mechanisms utilized by kinase ribozymes to catalyze biosynthetic reactions.

271-B The catalytic mechanism of a novel, widely disseminated small nucleolytic ribozyme*Timothy Wilson¹, Tomas Fessl¹, David Lilley¹*¹University of Dundee

The twister ribozyme, recently discovered in the Breaker laboratory, is a novel small nucleolytic ribozyme that is widely disseminated in the genomes of bacteria and eukarya (Roth and Breaker, personal communication). At its simplest, the ribozyme consists of one terminal and two internal loops joined by short helices and stabilized by two pseudoknots. Cleavage occurs in the internal loop distal to the terminal loop. Like other nucleolytic ribozymes, cleavage occurs via an internal phosphoester transfer reaction, resulting in 2'-3'-cyclic phosphate and 5'-OH termini. Metal ions do not appear to participate directly in the reaction as the ribozyme is active in a wide range of divalent and monovalent cations. The reaction is relatively independent of pH above pH 6, with activity declining with pH below this point.

We are investigating the importance of ten highly conserved nucleotides to the structure and activity of the ribozyme. All ten lie in the terminal and cleavage site loops, which are thought to interact to form the active ribozyme. Mutation reduces activity by one to three orders of magnitude under standard conditions and generally perturbs folding significantly as revealed by in-line probing. Two of these nucleotides are particularly interesting. Replacing the highly conserved C in the terminal loop with a U results in a 2000-fold loss in activity at pH 7, but the effect is ameliorated at high pH. Similarly, cleavage is 50-fold slower when the conserved G in the cleavage site loop is replaced by an A, with higher activity observed at lower pH. These effects are consistent with a catalytic mechanism in which the C and G participate in general acid-base catalysis and we are investigating this hypothesis through atomic mutagenesis and phosphorothiolate acid-rescue experiments. We will present our latest data and compare the proposed mechanism to those of other small nucleolytic ribozymes.

RNA structure and folding

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 274 B – 295 B

Location: Main Hallway & Sanada Foyer

- 274 B** **Sizing large RNA molecules using single molecule fluorescence correlation spectroscopy**
- 277 B** **A revised folding pathway of group II introns: Assigning specific structures to the individual FRET states**
- 280 B** **The plasticity of a structural motif in RNA: structural polymorphism of a k-turn in response to its environment**
- 283 B** **Metal ions determine the kinetics and thermodynamics of single RNA-RNA associations according to the Irving-Williams series**

- 286 B** **One-bead coarse grained models for RNA dynamics and folding**
- 289 B** **Molecular crowding accelerates ribozyme docking and catalysis**
- 292 B** **Structural complexity of Dengue untranslated regions: the role of cis-acting RNA motifs and pseudoknot interaction in modulating functionality of the viral genome.**

- 295 B** **The regulatory significance of RNA secondary structure in Arabidopsis**

274-B Sizing large RNA molecules using single molecule fluorescence correlation spectroscopy*Alexander Borodavka¹, Roman Tuma¹, Peter Stockley¹***¹Astbury Centre for Structural Molecular Biology, University of Leeds**

Large RNA molecules (>1kb), encompassing mRNA, long non-coding RNAs (lncRNA) and genomes of RNA viruses, play important role in many biological processes. However, given their size and general propensity of ssRNA to form extensive secondary structure it is difficult to study their folding. Single molecule fluorescence correlation spectroscopy (smFCS) allows to determine hydrodynamic size of large RNA molecules at concentrations approximating infinite dilution conditions (<1 nM), thus minimizing artifacts attributed to non-specific interactions and aggregation. We have labelled representative RNAs at 3'-end with a single fluorophore and examined their hydrodynamic sizes under low-salt conditions, i.e. in 'good solvent', when electrostatic interactions dominate and prevent formation of tertiary structure. Under these conditions most RNAs adopt an ensemble of relatively compact conformations. The average hydrodynamic radii can be related to the ensemble of branched structures generated by Mfold, suggesting that under low salt conditions the size of large RNAs is determined by electrostatic repulsion between partially double stranded, branched segments. The size of most large RNA molecules decreases in the presence of physiological concentrations of polyvalent cations, reflecting further folding into compact tertiary structure. In contrast to mRNAs and viral genomes, lncRNAs do not undergo similar compaction, maintaining their rather elongated shape. Such distinct shape may be related to their function as molecular scaffolds in gene regulation.

277-B A revised folding pathway of group II introns: Assigning specific structures to the individual FRET states*Erica Fiorini*¹, *Carlo Lucia*³, *Danny Kowanko*¹, *David Rueda*², *Roland K.O. Sigel*¹¹University of Zurich, Institute of Inorganic Chemistry, Winterthurerstrasse 190, Zurich, Switzerland; ²Imperial College London, Section of Virology, Department of Medicine, London W12 0NN, UK; ³University of Zurich, Institute of Inorganic Chemistry, Winterthurerstrasse 190, Zurich, Switzerland; University of Birmingham, Institute of Biomedical Research, Birmingham, B15 2TT, UK

Sc.ai5G is a large (~900 nts), multi-domain group II intron RNA transcribed from the mitochondrial genome of *S. Cerevisiae*. [1] These RNAs catalyze their self-excision upon formation of long-range tertiary interactions. Their folding and related catalytic activity are influenced by co-factors such as metal ions (especially Mg^{2+}) and proteins. We have designed a shortened construct of *Sc.ai5G*, D135-L14, labeled with the Cy3-Cy5 fluorophore pair and biotin for surface immobilization. This construct enables a detailed characterisation of the folding pathway by smFRET using TIRF microscopy, since it preserves the dynamics and the catalytic activity of the parent ribozyme. [2] Using smFRET spectroscopy, it becomes possible to record data from one single molecule isolated from its neighbors in real-time and the direct analysis of individual folding pathways of different molecules within a large ensemble. Previous studies revealed a linear three-state folding pathway from the unfolded to the native state devoid of kinetic traps. [2] The rate constants of those transitions depend on concentration and the type of metal ions (*i.e.* Mg^{2+} and Ca^{2+}). [2, 3] Our studies revealed an additional folding step. Furthermore we made three distinct mutants each carrying a different mutation that interrupts a specific tertiary interaction, known to be involved in crucial interdomain tertiary interactions (Figure 1). This enabled us to assign each FRET state to a specific folding intermediate. The construct carrying a tetra-loop mutation in Domain 5, involved in the ζ - ζ' interaction with D1 (D13mut5-L14), basically stops the folding process at the lowest FRET state. A 2-point mutation in Domain 3 aimed to interrupt the μ - μ' (D3-D5) docking (D15mut3-L14) does not have a drastic effect. In contrast, Domain 3 deletion (D15-L14) causes misfolding of the molecule to a more compact state (Figure 1). Moreover, the presence of the substrate affects the distribution of conformational intermediates and their dynamic equilibrium in the different mutants.

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Financial support by the European Research Council (to RKOS) and the University of Zurich is gratefully acknowledged.

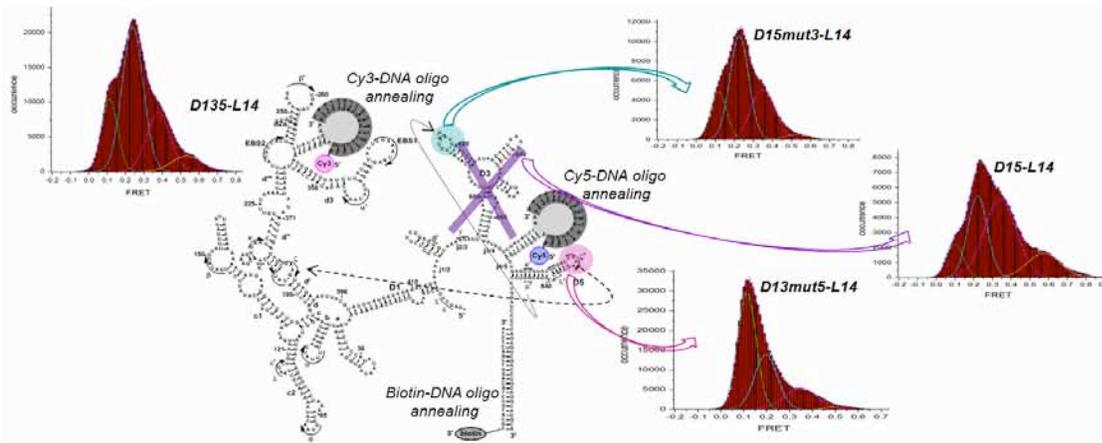


Figure 1. Secondary structure of labeled D135-L14 ribozyme (left). Mutations are indicated in green (D15mut3-L14), purple (D15-L14) and pink (D13mut5-L14). Histograms allow us to identify the number of conformations adopted by the molecule, the relative abundance of each FRET state and therefore the differences between the mutant constructs (right) and D135-L14 (left).

280-B The plasticity of a structural motif in RNA: structural polymorphism of a k-turn in response to its environment*Lin Huang¹, Peter Daldrop¹, David Lilley¹***¹CR-UK Nucleic Acid Structure Research Group, The University of Dundee**

The k-turn is a widespread structural motif that introduces a tight kink into the helical axis of double-stranded RNA, and is frequently used to make tertiary interactions. The adenine bases of consecutive G•A pairs are directed towards the minor groove of the opposing helix, hydrogen bonding in a typical A-minor interaction. We find that the available structures of k-turns divide into two classes, depending on a key A-minor hydrogen bonding in the core of the structure. The two classes differ on whether N3 or N1 of the adenine at the 2b position accepts a hydrogen bond from the O2' at the -1n position. There is a coordinated structural change involving a number of hydrogen bonds between the two classes.

We show that Kt-7 can adopt either the N3 or N1 structures depending on environment. While it has the N1 structure in the ribosome, we find that crystal structures of the same sequence, either engineered into the SAM-I riboswitch or in complex with the L7Ae protein as a simple duplex, have the N3 structure. The change between the N1 and N3 structures results in a significant alteration in the trajectory of the helical arms, and in the rotational setting of the helical arms. This will be very significant for making tertiary contacts.

283-B Metal ions determine the kinetics and thermodynamics of single RNA-RNA associations according to the Irving-Williams series

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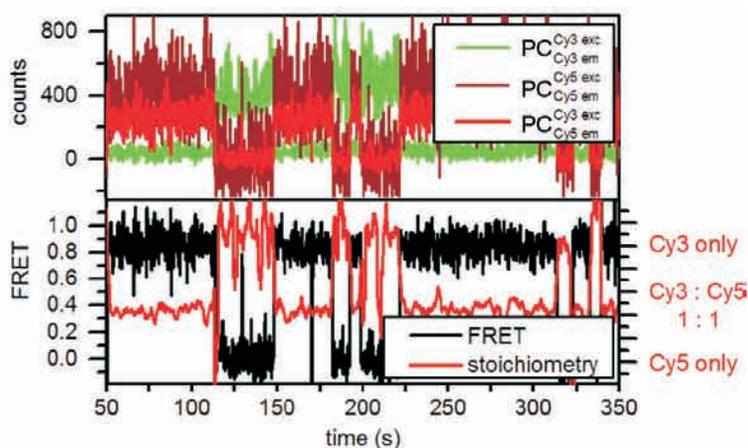
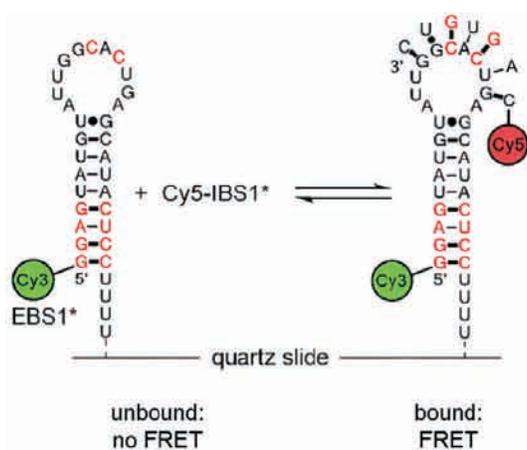
The formation and stability of a complex RNA architecture is to a large part dependent on base pairing, being the key to secondary and tertiary structure. Any such RNA-RNA interaction is only possible in the close proximity of cations, i.e. largely metal ions, to overcome charge repulsion of the negatively charged phosphate sugar backbone. Here we dissect the fundamental role of M^{2+} ions on the kinetics and thus also stability of RNA-RNA strand association. Here, we investigate the 5'-splice site formation of group II introns representing a typical example of such a basic interaction. Group II introns are functional RNAs that catalyze their own excision, i.e. splicing [1]. Splice site recognition is provided by the association of the exon- and intron-binding sequences (EBS, IBS), which has been proposed to be directly dependent on divalent metal ions [2]. We now used single-molecule FRET [3] to systematically assess the influence of various divalent metal ions along the Irving-Williams Series on the EBS1*/IBS1* interaction (Figure 1): In the rigorous absence of M^{2+} ions, strand pairing is basically absent, i.e. the presence of M^{2+} is crucial for EBS1*/IBS1* interaction to occur to a significant extent. However, the inter-oligonucleotide affinity as well as docking/undocking kinetic varies by several orders of magnitude depending on the cation present. These observations can be rationalized by the intrinsic affinities of the individual metal ions towards the different coordinating atoms, e.g. ring nitrogens, carbonyl oxygens, and phosphate oxygens, of the RNA. This allowed us to dissect such a "simple" RNA-RNA strand association into subsequent small steps of a clearly M^{2+} -dependent EBS1*/IBS1* folding pathway. Our study thus not only validates smFRET for the systematic study of metal ion-mediated nucleic acid folding, but also shows that metal ion-dependent RNA folding can be explained by the coordination chemistry of the metal ion cofactor.

Financial support by the European Research Council (ERC starting grant MIRNA 259092 to R.K.O.S.), a UZH-Forschungskredit (57010302, to S.L.B.K.), and the University of Zurich is gratefully acknowledged.

[1] O. Fedorova, N. Zingler *Biol. Chem.* **2007**, *388*, 665-678.

[2] D. Kruschel, R. K. O. Sigel *J. Inorg. Biochem.* **2008**, *102*, 2147-2154.

[3] L. Cardo, K. S. Karunatilaka, D. Rueda, R. K. O. Sigel, *Methods Mol. Biol.*, **2012**, *848*, 227-252.



286-B One-bead coarse grained models for RNA dynamics and folding

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The knowledge of RNA tertiary structure¹ and its internal dynamics² is essential for understanding key biological processes such as the regulation of mRNA translation. However, experimental studies of RNA structure and dynamics are expensive and troublesome. This is confirmed by a large discrepancy between the number of protein and RNA X-ray crystallography resolved structures in the Protein Data Bank. On February 19, 2013 there were more than 70 000 deposited protein X-ray structures and only 500 RNAs. In principle, computational approaches could affordably complement experiments. At this moment, however, the quality of RNA simulation methods and their predictive power needs improvement. One of the promising approaches is molecular dynamics with a coarse-grained representation of RNA. Nucleic acids are modeled as sets of beads and each bead corresponds to a single nucleotide. The time-dependent behavior of such beads is simulated by solving the classical equations of motions. This requires the formula for the potential energy function with parameters - the so called force field. Finding such a force field for various coarse-grained models of RNA is a long process, so a lot of effort has been put to find a universal force field that can be applied to all kinds of problems. However, in our opinion, the best results with a coarse-grained approach can only be obtained by carefully adapting the energy function to a particular need.

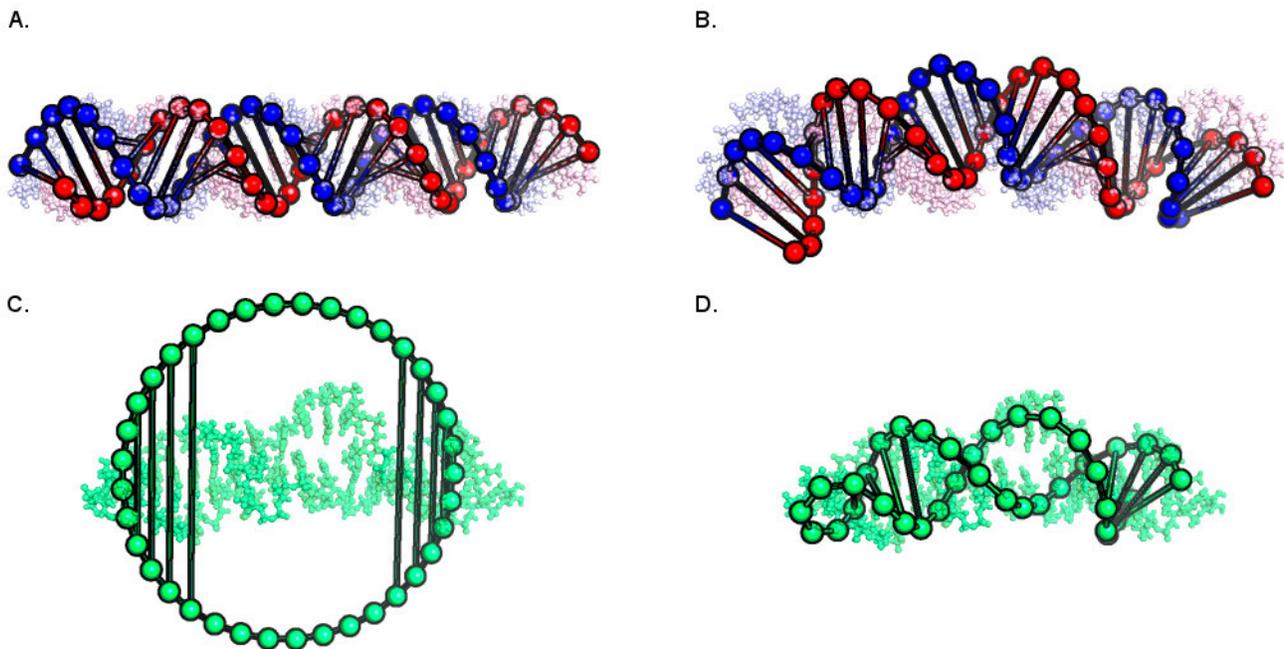
Therefore, we have created a methodological help for developers of coarse-grained models^{3,4}. We have implemented a metaheuristic optimization algorithm (evolutionary algorithm) that finds a best set of parameters for a given one-bead RNA potential energy function. Using such an automatized routine, in short time, one can adapt a general model to a particular task. As an example, we will show the results of the same one-bead coarse-grained force field, with parameters optimized in the first case for the internal dynamics of a folded RNA helix (Figure 1A,B) and in the second case for the RNA structure prediction (Figure 1C,D). We will present the differences in the construction of the two models that are essential for their performance for a particular task. We will also show statistical studies performed on thousands of force field models that emphasize the importance of particular force field terms and their relative correlations. The results allow us to define the boundaries of one-bead representations in modeling various aspects of RNA structure and dynamics.

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2. H. M. Al-Hashimi and N. G. Walter, *Curr. Opin. Struct. Biol.* 2008, **18**:321–329

3. F. Leonarski, F. Trovato, V. Tozzini, J. Trylska, *Lect. Notes Comp. Sci.* 2011, **6623**:147–15223

4. F. Leonarski, F. Trovato, V. Tozzini, A. Les, J. Trylska, in preparation for *J. Chem. Theory Comput.*



289-B Molecular crowding accelerates ribozyme docking and catalysis*Bishnu P. Paudel*¹, *David Rueda*²¹Imperial College, London / Wayne State University, Detroit MI; ²Imperial College, London, UK

All biological processes take place in highly crowded cellular environments. However, how molecular crowding agents affect the folding and catalytic properties of RNA molecules remains largely unknown. Here, we have combined single-molecule fluorescence resonance energy transfer (smFRET) and bulk cleavage assays to determine the effect of a molecular crowding agent (polyethylene glycol, PEG) in the folding and catalysis of a model RNA enzyme, the hairpin ribozyme. Our single-molecule data reveal that PEG favors highly the formation of the docked (active) structure by increasing the docking rate constant specifically with increasing PEG concentrations. Furthermore, in the presence of PEG, the concentration of Mg²⁺ ions required to induced the active state decreases by 10-fold, near the physiological range (~1 mM). Lastly, bulk cleavage assays show that the ribozyme's activity accelerates by ~10-fold in the presence of the crowding agent. Together, our data show that molecular crowding agents can affect both the dynamics and function of RNA enzymes, such as the hairpin ribozyme. We propose that crowding agents in the cell play an important role in stabilizing and accelerating the native structure of RNA enzymes *in vivo*.

292-B Structural complexity of Dengue untranslated regions: the role of cis-acting RNA motifs and pseudoknot interaction in modulating functionality of the viral genome.

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¹RT Biochemistry Section, HIV Drug Resistance Program, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA; ²CCR Nanobiology Program, Computational RNA Structure Group, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA; ³Department of Microbiology and Immunology, Georgetown University School of Medicine, Washington, D C 20057, USA

Dengue virus (DENV) is a single-stranded positive-sense RNA virus belonging to the *Flaviviridae* family, whose members cause diseases of major health importance. The DENV genome encloses multiple *cis*-acting elements required for translation and replication. Previous studies indicated that a 719 nt subgenomic minigenome (DENV-MINI) is an efficient template for translation and (-) strand RNA synthesis *in vitro* (1, 2). We performed a detailed structural analysis of DENV-MINI RNA, combining chemical acylation techniques (SHAPE, aiSHAPE), Pb²⁺ ion-induced hydrolysis and site-directed mutagenesis. Our results highlight protein-independent 5'-3' terminal interactions involving hybridization between specific *cis*-acting RNA motifs. Probing analyses identified tandem dumbbell structures (DBs) within the 3' terminus spaced by single-stranded regions, and internal loops and hairpins with embedded GNRA-like motifs. Analysis of conserved motifs and top loops (TLs) of these dumbbells, and their predicted interactions with downstream pseudoknot (PK) regions, predicted an H-type pseudoknot involving TL1 of the 5' DB and the complementary region, PK2. Since disrupting the TL1/PK2 interaction, via "flipping" mutations of PK2, previously attenuated DENV replication, this pseudoknot may participate in regulation of RNA synthesis. Computer modeling implied that this motif might function as autonomous structural/regulatory element. In addition, our studies targeting elements of the 3' DB and its complementary region PK1 indicated that communication between 5'-3' terminal regions strongly depends on structure and sequence composition of the 5' cyclization region.

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295-B The regulatory significance of RNA secondary structure in Arabidopsis*Lee Vandivier*¹, *Fan Li*⁴, *Qi Zheng*³, *Matthew Willmann*³, *Ying Chen*⁴, *Brian Gregory*²¹Genetics and Gene Regulation Graduate Group, PENN Genome Frontiers Institute, University of Pennsylvania;²Department of Biology, PENN Genome Frontiers Institute, Cell and Molecular Biology Graduate Program, Genomics and Computational Biology Graduate Program, University of Pennsylvania; ³Department of Biology, PENN Genome Frontiers Institute, University of Pennsylvania; ⁴Genomics and Computational Biology Graduate Program, PENN Genome Frontiers Institute, University of Pennsylvania

Recent transcriptome-wide studies have illustrated the role of RNA secondary structure as a post-transcriptional *cis*-regulator that controls multiple steps of mRNA maturation and translation. We have focused our attention on uncovering the regulatory impact of secondary structure on the *Arabidopsis thaliana* transcriptome. Here, we present findings from these recent studies revealing that mRNA structure modulates translation. For instance, we find that RNA secondary structure sharply decreases across translation start and stop sites, while increasing folding has significant positive correlation with ribosome occupancy, perhaps due to ribosomal stalling. We also demonstrate a correspondence between mRNA and protein structure. Specifically, the portions of mRNAs encoding predicted protein domains are on average significantly more structured than portions encoding inter-domain regions. We then reveal that RNA folding significantly anticorrelates with transcript abundance, due in part to increased degradation and smRNA processing of highly structured protein-coding mRNAs. Finally, we uncover that transcripts with similar structure not only share similar regulation, but also encode proteins with coherent functions. In total, we demonstrate the global impact of structure on post-transcriptional regulation in *Arabidopsis*.

RNA chemistry

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 298 B – 304 B

Location: Main Hallway & Sanada Foyer

298 B **Synthesis of fluorophore- and spin-labeled RNA using deoxyribozymes**

301 B **Translational regulation by ligand-inducible formation of RNA pseudoknot**

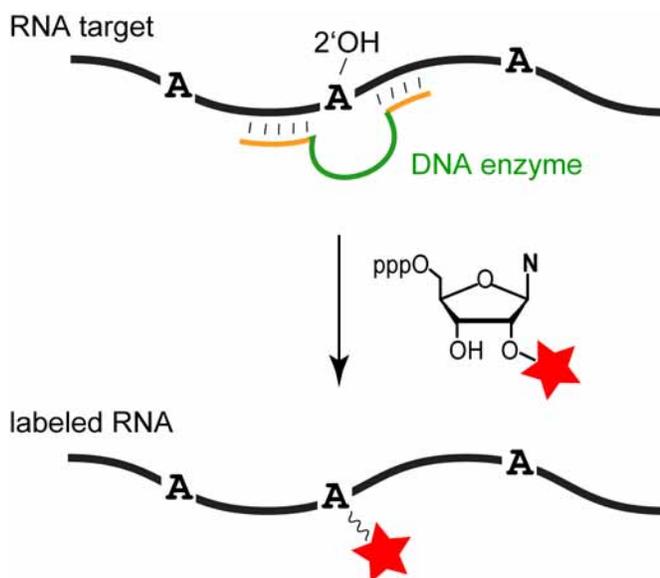
304 B **Chemical Synthesis of 3'-Aminoacyl-tRNA Mimics to Investigate Antibiotic Induced Ribosome Stalling**

298-B Synthesis of fluorophore- and spin-labeled RNA using deoxyribozymes*Lea Büttner¹, Fatemeh Javadi-Zarnaghi¹, Claudia Höbartner¹*¹Max Planck Institute for biophysical Chemistry, Research Group Nucleic Acid Chemistry, 37077 Göttingen, Germany

Studying the multifaceted functions of non-coding RNAs by biophysical techniques such as electron paramagnetic resonance (EPR) spectroscopy and fluorescence measurements requires the site-specific installation of spectroscopic labels. Several synthetic approaches are known to directly incorporate modifications via solid-phase synthesis or install reporter groups post-synthetically on pre-functionalized RNA. The investigation of longer RNAs than routinely achievable by chemical synthesis demands the ligation of labeled RNA fragments, which is traditionally performed with (protein) enzymes T4 DNA ligase or T4 RNA ligase.

Here we report on an alternative approach for obtaining long labeled RNAs by employing DNA enzymes (deoxyribozymes) for the site-specific attachment of reporter groups, as well as for the efficient ligation of labeled RNA fragments. Deoxyribozymes are artificial single stranded DNA molecules that are identified by in vitro selection and serve as useful tools for practical applications. Synthetically beneficial DNA enzymes require only metal ions (usually Mg^{2+} or Mn^{2+}) as cofactors for their catalytic activity, function at neutral pH and can easily be isolated and reused for multiple reactions. For the site-specific, posttranscriptional labeling of RNA, we capitalized on a DNA enzyme that has been shown to accept GTP as substrate to attach a single nucleotide to the branch site adenosine of a model RNA.¹ We have optimized this reaction based on our recent finding of lanthanide-assisted acceleration of DNA-catalyzed RNA ligations. In this report, we demonstrate the attachment of chemically modified nucleotide analogs that introduce bioorthogonal functional groups or directly install detectable probes at desired target sites (see Figure). In a general sense, this deoxyribozyme combines in one molecule the functions of target-site selection and catalysis of the labeling reaction. Guidelines for the general application of the deoxyribozyme-based labeling strategy are developed and the approach is exemplified for the synthesis of spin-labeled and fluorescently labeled *S*-adenosylmethionine (SAM)-binding riboswitch RNAs.

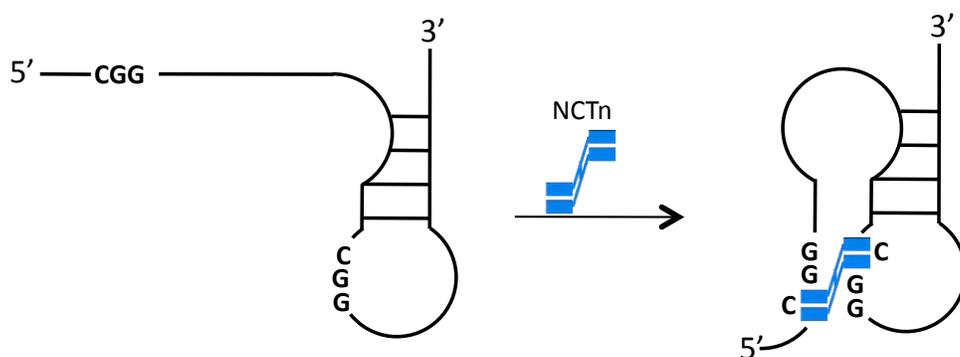
1. C. Höbartner, S.K. Silverman, *Angew. Chem. Int. Ed.* **2007**, *46*, 7420-7424.



301-B Translational regulation by ligand-inducible formation of RNA pseudoknot*Saki Matsumoto¹, Changfeng Hong¹, Asako Murata¹, Kazuhiko Nakatani¹*¹The Institute of Scientific and Industrial Research, Osaka University

RNA secondary structures play crucial roles for noncoding RNAs in catalyzing biological reactions, controlling gene expression, responding to cellular signals, and so on. A small molecule that binds specifically to target RNA and induces a particular secondary structure might be a promising tool for gene regulation and gene therapy. We have developed a series of synthetic small molecules that can specifically bind to G-G mismatches in double-stranded DNA. Naphthyridine carbamate tetramer (NCTn), one of such mismatch binding molecules, consists of four naphthyridine units connected by flexible methylene linker $-(CH_2)_n-$ and selectively binds to the CGG/CGG triad in dsDNA by forming hydrogen bonding with guanine bases. Our previous study has shown that NCTn can bind to $(CGG)_n$ sequence in single-stranded region of RNA and act as a molecular glue for promoting duplex formation from two single strand. Here we demonstrated that NCTn can induce a pseudoknot structure in RNA by the simultaneous binding between the loop region and the single-stranded tail as illustrated in Figure and furthermore, ligand-inducible formation of RNA pseudoknot can regulate translation of target gene by frame shifting.

We used VPK pseudoknot, which causes ribosomal frameshifting in mouse mammary tumor viruses, as the structural template for engineering to NCTn-inducible pseudoknots. Ribosomal frameshifting is the translational recoding mechanism used in many viruses, which is promoted by a heptanucleotide slippery sequence and an adjacent mRNA secondary structure, most often an mRNA pseudoknot. The ribosome is stalled at a pseudoknot and forced to shift one nucleotide backwards into an overlapping reading frame and to translate an entirely new sequence of amino acids. We designed NCTn-inducible frameshifting system by introducing a potential binding site (CGG/CGG triad) for NCTn in VPK sequence (mmVPK) located between the initiation codon and the firefly luciferase gene. The frameshifting efficiency increased by NCTn-induced pseudoknot folding can be detected by increase in luciferase activity. To evaluate the effect of NCTn on translation of the firefly luciferase gene, *in vitro* protein synthesis assay was performed. In the presence of NCT8, frameshifting efficiency gradually increased in a concentration-dependent manner, whereas only slight increase of frameshifting efficiency was observed without NCT8. Similar results were obtained for other NCTn ligands. These results suggested that NCTn could induce pseudoknot formation and regulate frameshifting efficiency. The detail of NCTn-induced frameshifting will be discussed.



304-B Chemical Synthesis of 3'-Aminoacyl-tRNA Mimics to Investigate Antibiotic Induced Ribosome Stalling

*Lukas Rigger*¹, *Shanmugapriya Sothiselvam*², *Nora Vázquez-Laslop*², *Alexander Mankin*², *Ronald Micura*¹

¹Institute of Organic Chemistry, Center for Molecular Biosciences Innsbruck (CMBI), Leopold Franzens University, Innsbruck, Austria; ²Center for Pharmaceutical Biotechnology, University of Illinois at Chicago, USA

Nascent proteins leave the ribosome through the peptide exit tunnel. For decades it has been thought that this tunnel plays a passive role in protein synthesis. Yet there are interactions between the nascent peptide and the ribosome that can lead to ribosome stalling. Such translation arrest is used for regulation of gene expression. Recent studies showed that the nature of the donor and acceptor substrates for the peptidyl transfer reaction plays an important role in the mechanism of programmed translation arrest [1]. To clarify whether charge or size of the aminoacyl moiety of the substrates is important for translation arrest, amino acids – natural and unnatural – covalently bound to RNA via hydrolysis-resistant linkages are required. Starting from orthogonally protected 3'-deoxy-3'-azido adenosine we synthesize functionalized solid supports for automated oligonucleotide-peptide synthesis [2]. With those in hand, we have efficient access to 3'-deoxy-3'-amide-linked aminoacyl-tRNA mimics. These conjugates represent important substrates for ribosomal stalling assays to shed light on the mechanism of translation arrest.

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Financial support from the Austrian Science Fund FWF (P21640, I317) is gratefully acknowledged.

Therapeutic RNAs

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 307 B – 313 B

Location: Main Hallway & Sanada Foyer

- 307 B** **CACNA2D4: a novel paradigm for the application of antisense-mediated gene therapy to the cure of retinal dystrophies**
- 310 B** **Identification of novel anti-obesity genes in primary human adipocytes using RNAi screening**
- 313 B** **mRNA transfection - a transient transgene expression in human mesenchymal stem cells as an attractive tool for regenerative medicine**

307-B CACNA2D4: a novel paradigm for the application of antisense-mediated gene therapy to the cure of retinal dystrophies

Niccolo Bacchi¹, Gian Carlo Demontis², Simona Casarosa¹, Michela Alessandra Denti¹

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Retinal dystrophies are a large set of genetic diseases that lead to partial or complete blindness as a consequence of retinal degeneration. No available cures have been reported so far. Antisense RNA-based correction approaches have important advantages over the extensively investigated gene therapy approaches. In particular, they would allow expression at the natural site and under physiological circumstances. The use of appropriately engineered small nuclear RNA-U1 (U1snRNA) can induce therapeutic "exon skipping" and restore gene expression impaired by different types of mutations. We are currently developing a U1snRNA based therapeutic approach for the cure of Autosomal Recessive Cone Dystrophy 4 (RCD4, OMIM #608171), caused by a mutation in the L-type calcium channel accessory subunit CACNA2D4. We will test the feasibility of this approach on *in vitro* (minigene systems, patients derived cells) and *in vivo* (mutant *Cacna2d4*^{S4804X} mice) models. The results of our work will foster current knowledge on RNA-based therapies, on their safety and on their effectiveness in the treatment of retinal dystrophies.

This work was supported by a "Giovani Ricercatori" Grant (GR-2008-1136933) by the Italian Ministry of Health.

310-B Identification of novel anti-obesity genes in primary human adipocytes using RNAi screening

Akanksha Gangar¹, Didier Grillot¹, Sandrine Martin¹, Valerie Linhart¹, Alizon Riou-Eymard¹, Raphaelle Guillard¹, Kim Carrein⁵, Maarten Van Balen⁵, Brigitta Witte⁴, Nick Vandeghinste⁵, Marlijn Steger², David Fischer², Steve De Vos⁵, Dominique Langin⁶, Vladimir Stich³, Stephane Huet¹

¹GlaxoSmithKline, 25-27 avenue du Québec, 91951 Les Ulis, France; ²BioFocus, Darwinweg 24, 2333 CR, Leiden, The Netherlands; ³Department of Sports Medicine, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic; ⁴Galapagos, Darwinweg 24, 2333 CR, Leiden, The Netherlands; ⁵Galapagos, Generaal De Wittelaan L11A3, 2800 Mechelen, Belgium; ⁶Inserm, UMR1048, Obesity Research Laboratory, Team 4, I2MC, Institute of Metabolic and Cardiovascular Diseases, Toulouse, France

Obesity is an epidemic health problem worldwide. It is characterized by excess fat deposit in the adipose tissue. An increase in adiposity is associated with a constellation of disorders known as the metabolic syndrome including insulin resistance, type 2 diabetes, hypertension and atherogenic dyslipidemia that contribute to the increased risk for cardiovascular morbidity & mortality. A multidisciplinary research effort involving a combination of clinical, biochemical and omics approaches appears mandatory to increase knowledge in the complexity of biological traits, processes associated with obesity and to identify novel targets for drug discovery.

We sought to identify novel genes implicated in adipose fat reduction by affecting triglyceride hydrolysis and/or increasing the metabolic capacity of the adipocytes. These gene products directly or components in the signaling pathway(s) of these gene products can be used as drug targets for the development of novel targeted anti-obesity therapies.

We employed loss-of-function RNAi genetic screen using an adenoviral shRNA library to identify genes involved in the reduction of adipose mass. The adenoviral shRNA library was generated against a set of target human genes known to be modulated in human overweight, obese or metabolic syndrome subjects. shRNA adenoviral transductions were performed in primary human sub-cutaneous adipocytes and five different read-outs (lipolysis, lipid droplet formation, adiponectin secretion, mitochondrial function and gene expression) were monitored. This was followed by confirmation of specific shRNAs to induce the desired phenotype by in-depth experimental analysis. Furthermore, "hits" were substantiated by correlation with the clinical parameters. This strategy allowed for the identification of several new genes involved in adipose functions along with some genes reported previously in published studies. We have reason to believe that some of these newly discovered genes may represent potential novel anti-obesity drug targets.

313-B mRNA transfection - a transient transgene expression in human mesenchymal stem cells as an attractive tool for regenerative medicine

*Adam Nowakowski*¹, *Anna Andrzejewska*¹, *Hanna Kozłowska*², *Piotr Walczak*⁴, *Barbara Lukomska*¹, *Mirosław Janowski*³

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²Advanced Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland;

³NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; Russell H. Morgan Dept. of Radiology and Radiological Science, Division of MR Research; Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, USA;

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⁴Russell H. Morgan Dept. of Radiology and Radiological Science, Division of MR Research; Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, USA

Introduction: Adult human bone marrow-derived mesenchymal stem cells (hMSCs) display a variety of beneficial properties. In stroke, therapeutic effects have been reported after the systemic delivery of hMSCs. It seems that a minimally invasive, intra-arterial route is an attractive method for stem cell transplantation to the injured brain. However, hMSCs lack the intrinsic mechanisms that make possible homing of the cells from the circulatory system to the area of infarction. It has been shown that genetic engineering can be effectively used for overexpression of certain molecules responsible for adhesion and transendothelial migration of systemically delivered cells. It is hypothesized that transient expression of integrins may be sufficient for promoting diapedesis and cell homing to the brain after intra-arterial delivery. Since hMSCs known for being extremely difficult to transfect using DNA plasmid vector, we investigated an mRNA transfection method for high efficiency expression of transgenes in hMSCs.

Methods: Human mesenchymal stem cells (hMSCs, PT-2501, Lonza) were cultured in a humidified atmosphere at 37°C and 5% CO₂ in an appropriate medium MSCBM (PT-3238, Lonza) supplemented with 10% MCGS (PT-4106E, Lonza), L-glutamine (PT-4107E, Lonza), and gentamicin sulfate (GA-1000, PT-4504E, Lonza). Cells were maintained in 75 cm² flasks but for transgene induction experiments were transferred to 24-well plates and seeded at a density of 15,000 cells/well. Plasmid DNA-eGFP (BD Biosciences) at a dose of 0.5 and 1.0 µg/well, and mRNA-eGFP (StemGent) at doses of 0.12, 0.25, and 0.5 µg/well were used. Four transfection agents, The Lipofectamine® 2000 (Invitrogen), TransIT-2020 (Mirus), TransIT® - mRNA (Mirus) and Stemfect™ RNA Transfection Kit (StemGent) were tested. The transfection efficiency was assessed over 21 days using GFP fluorescent signal detection by confocal microscopy.

Results: Consistently with previous reports the efficiency for pDNA-eGFP transfections of hMSCs was very low, less than 1%. In contrast, mRNA-eGFP transfection resulted in an efficiency exceeding 95% in all of the tested conditions. mRNA-eGFP dose of 0.5µg/well and the use of Lipofectamine® 2000 was the most effective method for transgene expression, lasting up to three weeks.

Conclusions: The mRNA transfection is an attractive tool for inducing transient expression of transgenes in otherwise difficult to transfect hMSCs. Due to its non-integration nature mRNA is highly desirable gene delivery technique for clinical applications. This engineering method for hMSCs may open new opportunities in regenerative medicine.

Supported by a National Centre for Research and Development grant No 101 in ERA-NET NEURON project: "MEMS-IRBI"

tRNA, snRNA, snoRNA, rRNA

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 316 B – 334 B

Location: Main Hallway & Sanada Foyer

- 316 B** Studies on structure-function relationships of the snoRNP assembly machinery
- 319 B** -High throughput quantification of tRNA function reveals unexpected interactions between tRNA residues
- 322 B** Biochemical Characterization of Archaeal RNase E-like Protein, FAU-1 in *Pyrococcus furiosus*
- 325 B** tRNAscan-SE and GtRNAdb: Improving Detection and Functional Prediction Based on Genomic Context, Structure, and Expression of Transfer RNAs
- 328 B** Thiolation of specific tRNAs by URM1 is required for efficient translation a subset of proteins by promoting ribosomal A-site binding
- 331 B** Human mitochondrial RNase P and its multiple faces
- 334 B** A Genome-wide Analysis to Identify Novel Genes Involved in tRNA Metabolism and Subcellular Trafficking

316-B Studies on structure-function relationships of the snoRNP assembly machinery

Christiane Branlant¹, Régis Back^{1,2}, Benjamin Rothé^{1,3}, Jonathan Bizarro⁵, Magali Blaud¹¹, Marc Quinernet⁴, Solange Morera⁸, Philippe Meyer⁷, Cyril Dominguez², Frédéric Alain³, Edouard Bertrand⁶, Xavier Manival¹⁰, Bruno Charpentier⁹

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As well pre-rRNA processing as pre-mRNA splicing depends upon activity of ribonucleoprotein particles. These RNPs are generated by association of sets of proteins onto small non coding RNAs, such as snoRNAs, scaRNAs or UsnRNAs. Work from the recent years demonstrated that like spliceosomal UsnRNP assembly, the assembly of snoRNPs which are involved in pre-rRNA processing and that of scaRNPs that are involved in UsnRNA modification depend upon cellular factors required to increase the efficiency and accuracy of assembly. We and others have identified an important factor of the snoRNP assembly machinery, the Rsa1(yeast)/NUFIP(human) protein. This platform protein associates with nascent RNPs by binding to the snoRNP RNA primary binding protein (Snu13 in yeast, 15.5K in human). Rsa1/NUFIP is able to bind to several other snoRNP core proteins and can recruit the R2TP complex a co-chaperon of the Hsp90 protein. The yeast R2TP complex contains proteins: Tah1, Pih1, Rvb1 and Rvb2. Tah1 forms a heterodimer with Pih1 and binds to Hsp90 by interaction with the Hsp90 C-terminal region. The idea is that by its numerous interactions with the core proteins and cellular factors, Rsa1 favors the specific recruitment of snoRNP proteins on the snoRNA and their remodeling, leading to stable mature snoRNPs. We focused our effort on understanding the mechanism of action and architecture of the C/D box snoRNP assembly machinery. We used yeast genetic approaches to determine the order of action of the cellular factors and identify their domains of interaction. In parallel, we developed structure-function analyses on components of the snoRNP assembly machinery based on NMR and other biophysical-chemical approaches. Studies on the R2TP-Hsp90 are as much important as this complex is involved in other assembly processes, in particular RNA polymerase assembly.

Although they play similar functions in snoRNP assembly, proteins Rsa1 and NUFIP show limited sequence homology. Only one 31 amino acid long sequence is strongly conserved. We showed that this peptide is crucial for the Rsa1p/NUFIP activity in box C/D snoRNP assembly. We identified an essential sub-fragment and solved its 3D structure by NMR. Then, by using the known 3D structure of yeast Snu13, we built a 3D model of the Rsa1-Snu13 interaction. We validated this model by biophysical methods and functional assays in yeast. In parallel, we purified Tah1 at a high degree of purity and established the 3D structure of free Tah1 and Tah1 bound to Hsp90. Our data explain how Tah1 specifically recognizes Hsp90. We characterized the interacting domains of Tah1p and Pih1p. We showed that Rsa1 bound to Snu13p can interact with another assembly factor Hit1 and also characterized the interacting domains. We will present the present stage of our functional and structural studies and their implication in terms of mechanism of action of the snoRNP assembly machinery.

319-B High throughput quantification of tRNA function reveals unexpected interactions between tRNA residues

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Evolution has fine-tuned tRNAs to allow for their efficient and accurate participation in translation, and much has been learned about the structural and functional roles played by many specific residues. However, it is not known precisely how each residue and modification contributes to the structure and function of tRNAs; nor is it known how much tolerance tRNAs have to mutation. Here we report a high throughput method to comprehensively quantify the effects of every possible single mutation and of many combinations of mutations on the function of an individual yeast suppressor tRNA, *SUP4_{ochre}* (*SUP4_{oc}*). Application of this method resulted in the identification of several unexpected interactions in tRNA.

We generated a library of 95,000 *SUP4oc* variants in which all residues (except for anticodon residues N₃₄-N₃₆ and residue N₃₇) contain random mutations at a 3% frequency. To quantify individual tRNA activity, we transformed the library into a strain carrying a *SUP4_{oc}*-suppressible codon in *GFP*, whose expression can be compared to a control *RFP* (Dean, K. M. and Grayhack, E. J. (2012) *RNA* **18**:2335-2344). Then, we sorted the *SUP4oc* transformants by fluorescence-activated cell sorting to separate cells into bins based on GFP/RFP expression, and analyzed the bins by recovering the *SUP4oc* genes and deep sequencing. A measure of the function of each variant tRNA is derived from the fractional representation of the sequence reads of the variant in each bin (Fowler, D. M., et al., (2010) *Nature Meth.* **7**:741-746). Individual analysis of 35 variants shows that experimentally determined GFP/RFP values measured by flow cytometry correlate well with quantification of function by deep sequencing.

These experiments yielded surprising results. First, we detected *GFP* suppression in 137 of 213 possible single mutant *SUP4oc* variants, 75 of which display more than 50% of wild-type activity. This result suggests that there is remarkable tolerance in the tRNA sequences that are acceptable for function. Second, we found a number of double mutant variants that are unexpectedly active given the activities of their constituent single mutants, and whose function is not easily explained by simple compensatory effects of residues that interact. We individually reconstructed and tested 12 such double mutant variants, and found that 11 of them recapitulated the data from deep sequencing. We will discuss possible explanations for these unexpectedly active variants, as well as our progress using this methodology to explore other important questions in tRNA biology, including the comprehensive analysis of determinants for the rapid tRNA decay pathway.

322-B Biochemical Characterization of Archaeal RNase E-like Protein, FAU-1 in *Pyrococcus furiosus**Yoshiki Ikeda¹, Asako Sato¹, Masaru Tomita¹, Akio Kanai¹*¹**Inst. Adv. Biosci., Keio Univ.**

Ribosomal RNAs (rRNAs) are important non-coding RNAs in all organisms. It is reported that precursor rRNAs (pre-rRNAs) are processed to mature rRNAs by various ribonucleases (RNases). RNase E is one of well-known endoribonucleases to process a pre-5S rRNA in *Escherichia coli*. However, in archaea, a processing enzyme of pre-5S rRNA has not been clearly understood yet. Previously, we identified an RNA-binding protein called FAU-1 that consists of 472 amino acids residues in the hyperthermophilic archaeon *Pyrococcus furiosus*. We showed that the N-terminal half of the FAU-1 had a degree of similarity (25%) with RNase E from *E. coli* (Kanai *et al.*, 2003). In the current research, we detected an endoribonuclease activity of the FAU-1 protein.

First, the recombinant FAU-1 protein with a His₆ tag sequence was induced in *E. coli*. Then, we purified the FAU-1 to near homogeneity by a His-affinity column chromatography, followed by a RESOURCE-Q ion exchange column chromatography. Using the purified protein, it was tested whether the FAU-1 was able to process *P. furiosus* pre-5S rRNA *in vitro*. As a result, accumulation of the FAU-1-dependent cleavage was observed by Northern blot analysis, and the cleavage site can be mapped at approximately 10 bases upstream of the 5'-end of mature *P. furiosus* 5S rRNA. A cleavage site specificity by the FAU-1 was also examined using a short RNA probe (73 nt) partially representing the *P. furiosus* pre-5S rRNA. The result showed that FAU-1 preferentially cleaved the AU-rich sequences of the RNA probe. Next, to confirm that FAU-1 actually possessed the RNase activity, we constructed a plasmid encoding mutant FAU-1 lacking 170-189 amino acid residues that showed a high degree of amino acid similarity among closely related proteins in archaeal species. Consequently, the mutant protein markedly reduced the FAU-1-dependent cleavage against the pre-5S rRNA, showing that the FAU-1 is an endoribonuclease and possibly involved in the pre-5S rRNA processing.

In previous study, it is reported that 5' end of the pre-5S rRNA is processed by tRNase Z in archaeon *Haloferax volcanii*, and transfer RNA (tRNA)-like structure in the 5' end of pre-5S rRNA is necessary to recognize by tRNase Z (Hölzle *et al.*, 2008). However, such tRNA-like structure has not been found in most of archaeal pre-5S rRNAs. Our results suggest that the FAU-1 could provide an alternative pathway to process the pre-5S rRNA in archaea.

325-B tRNAscan-SE and GtRNAdb: Improving Detection and Functional Prediction Based on Genomic Context, Structure, and Expression of Transfer RNAs

Todd Lowe¹, Patricia Chan¹, Aaron Cozen¹, Andrew Holmes¹, Jay Kim¹, Eva Robinson¹

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Transfer RNAs (tRNAs) are the largest, most complex non-coding RNA family that is universal to all living things. tRNAs are central to the flow of genetic information from messenger RNAs to proteins, and as ancient molecules, have acquired or retained a variety of other distinct functions in the cell (1, 2). The breadth and complexity of these functions are still coming into focus, but new hints are emerging from the study of genomic context, atypical structural variants, and differential expression/ChIP-seq patterns observed for various subsets of tRNAs. While there are now other useful, specialized tRNA detection methods available, tRNAscan-SE (3) and the Genomic tRNA Database (4) continue to be the most common source of gene predictions. Over the past decade, we have collected a large number of feature requests and algorithmic improvements that we are now actively implementing. These improvements will enhance the ability of tRNAscan-SE to more accurately identify and classify both typical and atypical tRNAs, identify partial tRNAs, and estimate biological relevance based on structural features, evolutionary conservation, and gene expression data. The GtRNAdb is being redesigned to integrate gene expression and other functional data, and automatically identify inconsistencies or unexpected features of full-genome gene sets. These analyses have already identified numerous outliers meriting closer study in vertebrate and microbial genomes. We present a variety of new observations from our work, including in-depth analyses of the human tRNA gene set.

We highly encourage tRNA researchers to contribute additional feature requests for tRNAscan-SE, as well as gene expression, population SNP variants, modification, and tRNA editing data to incorporate into the improved GtRNAdb (lowe@soe.ucsc.edu).

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328-B Thiolation of specific tRNAs by URM1 is required for efficient translation a subset of proteins by promoting ribosomal A-site binding

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Faithful protein synthesis relies on correct and efficient recognition of codons by tRNA molecules. To ensure this key role, tRNA molecules undergo many different modifications. In particular the wobble position, nucleotide 34, of the anti-codon is a hot spot for modifications and is thought to be functionally important for translation efficiency and fidelity. The wobble Uracil of tRNA^{LYS}_{UUU}, tRNA^{GLU}_{UUC}, and tRNA^{GLN}_{UUG} is universally modified to 5-methyl-2-thio derivatives. Specifically, in eukaryotes these tRNAs bear the 5-methoxycarbonyl-methyl-2-thio modification (mcm⁵s²). Previous *in vitro* studies have implicated this modification in modulating the wobble capacity of these tRNAs, however the exact *in vivo* function of this modification has been largely unexplored. In *Saccharomyces cerevisiae*, the *URM1*-pathway is responsible for tRNA thiolation at the Uracil-34 and is important for resistance to various stresses such as nutrient starvation and oxidative agents.

In this study, we show that thiolation is not required for general translation but rather regulates translation of a subset of mRNAs. We performed a proteome wide analysis to identify changes in protein abundance induced by lack of thiolation. Subsequent bioinformatic analysis revealed that tRNA thiolation is required for the efficient translation of a subset of mRNAs rich in the cognate AAA, CAA, and GAA codons. Further analysis of selected candidates revealed that the decreases in protein abundance were not mediated by differential transcription nor degradation and therefore likely results from differential translation. Codon usage and translation regulation was assessed *in vivo* using a codon-specific fluorescent translation reporter in single cells. Indeed, we found that expression of translation reporters enriched for AAA, CAA or GAA codons was impaired in cells lacking *URM1*, while reporters enriched for the synonymous AAG codons showed wild-type expression levels. Furthermore, *in vitro* studies using native tRNAs from yeast showed that thiolation enhances ribosomal A-site binding and thereby increases peptide bond formation rates.

Taken together our data, show that tRNA thiolation at the wobble position is important to control expression of a subset of mRNAs rich in AAA, CAA, and GAA codons and that this is mediated by increased A-site binding to the ribosome. We suggest a model in which tRNA modification regulates expression of a subset of mRNAs enriched for specific codons under stress conditions.

331-B Human mitochondrial RNase P and its multiple faces*Elisa Vilardo¹, Nadia Brillante¹, Christa Nachbagauer¹, Walter Rossmannith¹*¹**Center for Anatomy & Cell Biology, Medical University of Vienna, Austria**

The mitochondrial genome is transcribed as long polycistronic precursor RNAs, encoding a complete set of tRNAs interspersed among rRNAs and coding RNAs. The processing of the tRNAs determines the concomitant release of all the RNAs required for mitochondrial protein synthesis. We have previously identified the human mitochondrial RNase P, the endonuclease responsible for 5' processing of tRNAs, which consists of three protein subunits. Moreover, we have shown that two of these subunits of RNase P constitute the methyltransferase responsible for methylation of purines at position 9, a modification supposed to be crucial for the proper folding of tRNAs. The human mitochondrial enzyme is unusual because of its ability to methylate both A and G at position 9 of tRNAs. Furthermore, in contrast to related methyltransferases, it includes as subunit a short-chain dehydrogenase, involved in the degradation of fatty and amino acids and thus with no obvious connection to tRNA maturation and function. The tRNA cleavage, tRNA methylation and dehydrogenase activities, physically associated in human mitochondrial RNase P complex, are nevertheless uncoupled and independent from each other. Thus, human mitochondrial RNase P is a multifunctional complex gathering diverse enzymatic activities related to tRNA maturation and beyond

334-B A Genome-wide Analysis to Identify Novel Genes Involved in tRNA Metabolism and Subcellular Trafficking*Jingyan Wu¹, Anita K. Hopper¹***¹Department of Molecular Genetics, Center for RNA biology, The Ohio State University, Columbus, OH, 43214, USA**

tRNAs are major components of the cell's protein synthesis machinery. In addition to this essential role in gene expression, they also contribute to other diverse functions including protein degradation, apoptosis, cellular response to stress, and tumorigenesis. tRNAs are transcribed in the nucleus. After the removal of the 5' and 3' ends and the addition of CCA and some modifications, tRNAs are exported to the cytoplasm where they complete their biogenesis and fulfill their functions. In both yeast and vertebrate cells, the subcellular movement of tRNAs involves the initial export of tRNAs from the nucleus to the cytoplasm, retrograde nuclear import of cytoplasmic tRNAs, and re-export of the imported tRNAs back to the cytoplasm. Although tRNAs have been studied for decades, some major players in tRNA metabolism and subcellular movement remain unknown. For example, there is an unknown nuclear export pathway for intron-containing tRNAs in yeast. The overall aim of my research is to identify and characterize all the missing gene products involved in tRNA biology, using yeast, *S. cerevisiae*, as a model organism. My strategy is to conduct a genome-wide assessment of the impact of every gene upon tRNAs utilizing the yeast deletion and temperature-sensitive (ts) collections. To conduct this screen in a timely fashion, I've developed a rapid method for genome-wide analysis of small RNAs from strains in the mutant collections. This method implements three optimized techniques: a procedure for growing small yeast cultures in 96-deepwell plates, a fast procedure for small RNA isolation from the plates, and a sensitive nonradioactive Northern method for RNA detection (Wu *et al.*, in press). To date, 3168 mutants in the deletion and ts collections have been analyzed; several candidates that affect tRNA biology have been identified and verified. For example, deletion of *GLN3* causes defects in the early steps of tRNA biogenesis. We hypothesize that Gln3, a well-characterized nitrogen-responsive transcription factor, plays a novel role in pre-tRNAs processing. This study will uncover important factors that function in tRNA metabolism and intracellular trafficking, which will contribute to a better understanding of the complexity of tRNA biology.

Ribosomes and Translation

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 337 B – 340 B

Location: Main Hallway & Sanada Foyer

- 337 B** **When p53 senses faulty ribosomes: Induction of Tp53 correlates with enhanced expression of c-Myc target nucleolar proteins in Rpl11-deficient zebrafish**
- 340 B** **Antibiotic-mediated frameshift during translation of the leader ORF reveals a new principle of gene regulation**
- 343 B** **Alu RNPs target 40S ribosomal subunits to repress translation initiation**
- 346 B** **Crystal structures of the protozoal cytoplasmic A site in complex with aminoglycosides**
- 349 B** **Molecular characterization of ribosomal E-site function**
- 352 B** **Effect of tRNA core modifications and a D-loop sequence element on ribosomal decoding**
- 355 B** **Molecular pathogenesis of ribosomopatias in zebrafish model for Diamond-Blackfan anemia**
- 358 B** **Ribosome biogenesis in plants and its impact on developmental processes**
- 361 B** **Towards understanding the inhibitory effects of codon-anticodon wobble base pairing on the ribosome**

337-B When p53 senses faulty ribosomes: Induction of Tp53 correlates with enhanced expression of c-Myc target nucleolar proteins in Rpl11-deficient zebrafish*Anirban Chakraborty¹, Tamayo Uechi², Pierre-Emmanuel Gleizes¹, Naoya Kenmochi²*¹CNRS, Laboratoire de Biologie Moléculaire Eucaryote (LBME), Toulouse, France; ²Frontier Science Research Center, University of Miyazaki, Japan

Nucleolar proteins play critical role in rRNA processing and pre-ribosomal assembly within the nucleolus. Impaired ribosome biogenesis causes nucleolar stress that triggers a p53 signaling pathway. Studies in cell lines have identified RPL11 as a key mediator in this pathway because of its ability to directly interact with MDM2, the negative regulator of p53. Interestingly, RPL11 has also been shown to control the transcriptional activity of c-Myc, an oncoprotein that positively regulates ribosome biogenesis. Contrary to the cell-line based results, we have previously demonstrated that loss of Rpl11 activates the Tp53 pathway in zebrafish. To gain further insight into the mechanism of Tp53 induction in response to Rpl11 deficiency, we analyzed the level of c-Myc and several c-Myc target nucleolar proteins in Rpl11-deficient zebrafish. Quantitative RT-PCR revealed 3-5 fold upregulation of c-Myc and the majority of its target nucleolar proteins, including those that bind to MDM2 or modify p53, in Rpl11-deficient zebrafish. *In situ* hybridization showed an enrichment of nucleolar protein transcripts in the head region of the Rpl11-deficient zebrafish, where morphological abnormalities and *tp53* localization were more pronounced. Furthermore, a time-course analysis indicated that the overexpression of nucleolar proteins coincided with the activation of Tp53 response in the Rpl11-deficient zebrafish. Rpl11 deficiency also led to defective rRNA processing and decreased abundance of functional ribosomes in zebrafish, indicating an impaired ribosome biogenesis. Many nucleolar proteins analyzed in this study are known p53 modulators. Therefore our results suggest a model in which increased c-Myc activity upon Rpl11 loss-of-function stimulates synthesis of nucleolar proteins, which, in turn, may trigger a p53 response.

340-B Antibiotic-mediated frameshift during translation of the leader ORF reveals a new principle of gene regulation

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The antibiotic-inducible gene *ermC* encodes an rRNA methyltransferase that renders bacteria resistant to macrolide antibiotics. The *ermC* gene is preceded by a 19 codon-long regulatory ORF. In the absence of antibiotic, the secondary structure of the intergenic region occludes the ribosome binding site and the initiation codon of the methyltransferase gene impeding its expression. The currently known classic mechanism of antibiotic-mediated induction of *ermC* expression operates via nascent peptide dependent ribosome stalling. A ribosome with bound erythromycin, an inducing macrolide antibiotic, stalls at a strategic location of the *ermCL* ORF and triggers mRNA isomerization. The conformational switch frees the *ermC* initiation region, allowing a drug-free ribosome to initiate expression of the resistance gene. Ribosome stalling critically depends on the sequence of the leader peptide and on the structure of the inducing macrolide antibiotic which binds to the ribosomal exit tunnel. Interestingly, the new generation of macrolides, the ketolides, which also bind in the exit tunnel, are unable to direct ribosome stalling at *ermCL*. Yet, puzzlingly, ketolide telithromycin can induce expression of *ermC*. We have unraveled the mechanism of ketolide-mediated induction of *ermC*. The novel mechanism uncovers a novel principle of gene regulation based on readthrough of the stop codon of the regulatory gene and reveals the previously unknown ability of macrolide drugs to cause translational frameshifting.

The mechanism of ketolide-mediated induction of *ermC* operates via antibiotic-induced -1 frameshift that takes place at the two last sense codons of the *ermCL* leader ORF. The N-terminal amino acid sequence of the ErmCL peptide allows it to thread through the antibiotic obstructed exit tunnel without displacing the drug. Consequently, the ribosomes with bound antibiotic reach the end of the ORF. The 'slippery sequence' encompassing the two last sense codons of *ermCL* and the presence of the antibiotic in the tunnel are the prerequisites for the frameshift to occur. After switching to the -1 frame, ribosomes can translate the entire intergenic region without encountering any stop codons until they reach the beginning of the *ermC* gene. Unfolding of the mRNA structure by translating ribosomes or re-initiation of translation likely facilitate expression of the resistance cistron. Our findings reveal a new paradigm of gene regulation by programmed frameshift at the regulatory ORF and open new venues for development of superior antibiotics.

343-B Alu RNPs target 40S ribosomal subunits to repress translation initiation*Elena Ivanova*¹, *Audrey Berger*¹, *Elena Alkalaeva*², *Anne Scherrer*¹, *Katharina Strub*¹¹Department of Cell Biology, University of Geneva, Switzerland; ²Engelhardt Institute of Molecular Biology, Moscow, Russia

In primate cells, non-coding cytoplasmic *Alu* RNAs transcribed from the repetitive *Alu* elements form complexes, referred to as *Alu* RNPs, with the protein heterodimer SRP9/14. SRP9/14 is also a part of the signal recognition particle (SRP), a ribonucleoprotein complex, which plays a key role in protein translocation into the endoplasmic reticulum. The SRP RNA gene is the phylogenetic precursor of the *Alu* elements. We found that biochemically purified *Alu* RNPs inhibit translation initiation in rabbit reticulocyte lysate on capped and non-capped mRNAs in a dose-dependent manner whereas the protein and the RNA moieties alone had no effect. The identity of the RNA moiety in the *Alu* RNP was important for function. RNP particles assembled on *Alu* RNA derived from the SRP RNA gene failed to inhibit initiation. Those containing RNAs from the old *Alu* J and the younger *Alu* Sx families of *Alu* elements produced an intermediated phenotype whereas RNPs assembled on *Alu* RNA from the youngest Y family were the most efficient in translation inhibition indicating that this function was acquired during evolution. In the protein moiety of the *Alu* RNP, the presence of a positively charged domain composed of the C-terminal pentapeptide in SRP14 and three lysines in SRP9 was required to inhibit translation initiation. To investigate the mechanism of inhibition, we studied the effect of *Alu* RNPs on the *in vitro* assembly of different translation complexes. We found that *Alu* RNPs interfere with 48S complex formation by inhibiting the recruitment of the mRNA to the 43S complex. Moreover, *Alu* RNP repressed ribosomal complex assembly on the cricket paralysis virus IRES which occurs independently of any initiation factors, suggesting that *Alu* RNPs has a direct effect on 40S subunit. Consistently, we found SRP9/14 to be bound to 40S subunits upon their incubation with *Alu* RNPs. Our results describe a novel translational control mechanism in which *Alu* RNA ensures functional binding of SRP9/14 to the 40S ribosome and thereby prevents cap-dependent as well as IRES-mediated ribosome recruitment to the mRNA. This pathway might be activated in response to viral infection to interfere with IRES-dependent translation initiation.

346-B Crystal structures of the protozoal cytoplasmic A site in complex with aminoglycosides*Jiro Kondo*¹, *Mai Koganei*¹¹**Sophia University**

Parasitic infections recognized as neglected tropical diseases are a source of concern for several regions of the world. Aminoglycosides are potent antimicrobial agents, which have been extensively studied by biochemical and structural studies in prokaryotes. However, the molecular mechanism of their potential antiprotozoal activity is less well understood.

In the present study, we have determined crystal structures of the protozoal cytoplasmic A site (aminoacyl-tRNA decoding site) in complex with aminoglycosides possessing a 6'-hydroxy group on ring I. They specifically bind within the deep/major groove of the protozoal cytoplasmic A site, where they displace two universally conserved adenines A1492 and A1493 (according to the numbering used in *E. coli* 16S rRNA), and stabilize the state called "on". Since two bulged-out adenines are involved in contacts with mRNA-tRNA complex, the binding of aminoglycosides to the protozoal cytoplasmic A site may lead to reduced discrimination of cognate tRNAs versus near-cognate tRNAs, thereby compromising translation fidelity.

Ring I of aminoglycosides with a 6'-hydroxy group stack on G1491, which is highly conserved in bacterial and protozoal species. In addition, ring I can form a pseudo pair with G1408 through two hydrogen bonds and one C-H...O bond. It is obvious from structural modeling that aminoglycosides with a 6'-ammonium group on ring I, such as gentamicin and kanamycin, cannot make the identical pseudo pair, because the ammonium group repels N1-H or N2-H of G1408. In fact, aminoglycosides with a 6'-ammonium group do not display antiprotozoal activity.

It is noteworthy that the secondary structure of the protozoal cytoplasmic A site is highly analogous to that of the bacterial A site with an A1408G mutation, which is the most prevalent antibiotic-resistant mutation found in clinical isolates. As evidence of this, the mutation is known to confer high-level resistance to aminoglycosides with a 6'-ammonium group but moderate resistance to those with a 6'-hydroxy group.

The structure information obtained in this study would contribute towards the structure-based design of next-generation aminoglycosides with high activities against parasitic protozoa and antibiotic-resistant bacteria.

349-B Molecular characterization of ribosomal E-site function*Nicola Rusca*¹, *Nina Clementi*², *Matthias Erlacher*², *Norbert Polacek*¹¹Department of Chemistry and Biochemistry, University of Bern, Bern, Switzerland; ²Innsbruck Biocenter, Division of Genomics and RNomics, Medical University Innsbruck, Austria

During the elongation cycle of protein biosynthesis, tRNAs travel through the ribosome by consecutive binding to the three ribosomal binding sites (A-, P-, and E-sites). While the A and P sites have been functionally well characterized in the past, the contribution of the E site to translation is still poorly understood in molecular terms. Footprinting and crystallographic studies indicated an interaction of A76 of E-tRNA with the nucleobase of the universally conserved 23S rRNA residue C2394.

We use the 'atomic mutagenesis' approach,^[a] which allows site-specific manipulation of functional rRNA groups in the context of reconstituted ribosomes, to identify critical interaction partners of E-tRNA with the 23S rRNA. The functional significance of E-site 23S rRNA nucleotides for protein synthesis was tested by performing *in vitro* translation reactions with chemically engineered ribosomes carrying either a deoxy-residue or an abasic nucleoside analog at position C2394, U1851, A2422, or G2421. Unexpectedly the removal of the nucleobase or the ribose 2'-OH at C2394 had no effect on protein synthesis. Also modifications at U1851 or A2422 had no inhibitory effect. However, removing the nucleobase at G2421 completely inhibited *in vitro* translation. G2421 forms a base pair with C2395 and it was shown that A76 of E-tRNA stacks on the nucleobase of G2421. Subsequent standard mutagenesis highlighted the importance of a classical Watson-Crick 2421-2395 base pair regardless of the involved nucleobase identities. Ongoing work focuses on the importance of this universally conserved base pair in the 50S E-site on E-tRNA binding, reading frame maintenance and translation accuracy.

^[a] Erlacher et al. (2011), Nat. Prot., 6:580-592.

352-B Effect of tRNA core modifications and a D-loop sequence element on ribosomal decoding*Irina Shepotinovskaya*¹, *Olke Uhlenbeck*¹¹Northwestern University, Evanston, IL USA

Unmodified tRNAs are capable of adopting the folded tertiary structure characteristic of native tRNAs, although they are less stable and more dynamic. While unmodified tRNAs are often active enzyme substrates, their activity on ribosomes may be compromised. tRNAs lacking all or some of the modifications in the anticodon stem loop (ASL) show weaker ribosome binding, slower rates of dipeptide bond formation and increased misreading than their modified forms.(1) Here we focus on the decoding properties of modified and unmodified *E. coli* tRNA Ala(GGC), tRNA Gly(GCC) and tRNA Val(GAC), which are the only three *E. coli* elongator tRNAs that naturally lack ASL modifications, permitting the combined effects of the less studied modifications in the core of tRNA to be evaluated.

Using ribosome decoding assays in a "high fidelity" buffer at 25°C, we confirm the results of our earlier experiments in RBB buffer indicating that the unmodified forms of all three of these tRNAs show identical rates of peptide bond formation (k_{pep}) as their native counterparts. However, at elevated temperatures (37°C and 42°C), native tRNA Ala and tRNA Gly show substantially faster k_{pep} values than at 25°C while the unmodified tRNAs remain slower. In-line probing experiments show that at 37°C the tRNA tertiary structure is partially disrupted when the modifications are absent, but not when the modifications are present. This conclusion is consistent with earlier studies indicating a stabilizing effect of the T54 and ?55 modifications (2).

Interestingly, the k_{pep} value of unmodified tRNA Val(GAC) remains equal to that of native tRNA Val(GAC) at all temperatures, suggesting that this tRNA does not need modifications to maintain its tertiary structure in the conditions tested. By measuring k_{pep} with a set of unmodified tRNA Val/ tRNA Gly chimeras, the source of the increased k_{pep} activity at higher temperatures was found to entirely be due to the presence of U20.1, which only occurs in tRNA Val. Removing U20.1 from unmodified tRNA Val makes it less active at 37°C, while adding U20.1 to tRNA Ala makes it more active at 37°C. In-line probing experiments confirm that the presence of U20.1 stabilizes the folded tertiary structure of tRNA.

A residue at position 20.1 (usually a U) is present in 40% of class 1 bacterial tRNAs. Although most tRNA species either have 20.1 or do not, a few such as tRNA Val(GAC) contain 20.1 in some bacteria and not in others. U20.1 is expected to be modified to D in many organisms. We are interested in understanding whether this residue acts as an independent stability element, why it is only present in certain tRNAs and how it acts to stabilize tRNA structure.

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355-B Molecular pathogenesis of ribosomopathies in zebrafish model for Diamond-Blackfan anemia*Tamayo Uechi*¹, *Yukari Nakajima*¹, *Hiroko Sonoda*³, *Masahiro Keda*³, *Yutaka Suzuki*², *Sumio Sugano*², *Naoya Kenmochi*¹¹Frontier Science Research Center, Univ. of Miyazaki; ²Dept. of Medical Genome Science, Univ. of Tokyo; ³Dept. of Veterinary Pharmacology, Univ. of Miyazaki

Ribosome biogenesis in eukaryotes involves a coordinated participation of ribosomal RNAs (rRNAs), ribosomal proteins (RPs), and a large number of accessory factors that contribute to processing of precursor rRNAs and assembly of ribosomal subunits. Recently, mutations in genes involved in ribosome biogenesis were identified in patients with various diseases, suggesting impaired ribosome can cause tissue-specific failures (ribosomopathies) via an unknown pathway. Diamond-Blackfan anemia (DBA) is one of such disorders, characterized by diminished numbers of erythroid progenitors. The DBA has been attributed to the defect in RP genes, such as *RPS19* and nine other so far. However, it is still unclear how mutations in such ubiquitously expressed genes affect mainly erythropoiesis.

To investigate the molecular mechanism underlying DBA, we have developed a zebrafish model for the anemia by repressing the translation initiation of *rps19* mRNA with the Morpholino antisenseoligo. The knockdown embryos displayed a drastic reduction of red blood cells, whereas differentiation of other myeloid cells and endothelial cells seemed to be normal. The anemic phenotype was almost completely rescued by injection of synthesized *rps19* mRNA, but not by mutated mRNAs with patient-type mutations. The DBA model also showed developmental abnormalities in the head and tail regions due to increased cellular apoptosis. A simultaneous inhibition of *p53* gene rescued the morphological abnormalities, but did not alleviate the erythroid aplasia. This suggests that a *p53*-independent but *RPS19*-dependent pathway could be responsible for defective erythropoiesis in DBA. To evaluate the impact of *RPS19* deficiency on translation, we carried out a polysome profiling of our DBA model. The polysome patterns were similar between *rps19* morphants and control embryos, although the amount of the heavier fractions was less in the morphants. Next, we carried out an RNA-Seq analysis of polysomal mRNAs purified from these embryos. We found that the translational efficiencies of about 300 genes were significantly changed in the *rps19* morphants. These data will provide important information to understand the pathogenesis of DBA.

358-B Ribosome biogenesis in plants and its impact on developmental processes*Benjamin Weis*¹, *Sandra Missbach*², *Enrico Schleiff*¹¹Molecular cell biology of plants, insitute for molecular biosciences, Goethe-University Frankfurt am Main, Germany;²Novartis Vaccines and Diagnostics GmbH, Marburg, Germany

Ribosomes are highly complex ribonucleoprotein particles responsible for the synthesis of all proteins. Eukaryotic ribosomes consist of 79 ribosomal proteins and four ribosomal RNA molecules. Three of the four ribosomal RNAs (rRNA) are transcribed as a polycistronic transcript that is processed by endo- and exnucleolytic digestions to release the mature rRNAs. The processing path is well understood in yeast, but there is only limiting information on rRNA processing in plants. Only recently, a number of cleavage sites were mapped using mutants of the Arabidopsis orthologs of yeast Rat1/Xrn2 and Mtr4. rRNA processing and folding are prerequisite for the assembly of eukaryotic ribosomes, which additionally requires up to 200 ribosome assembly cofactors and more than 75 snoRNAs. Interestingly, In the plant model organism *Arabidopsis thaliana*, 2/3 of the ribosome biogenesis cofactors, that are orthologs of the yeast cofactors, are found multiple times in the genome pinpointing to additional or redundant functions of these factors. Furthermore, the different orthologs might be required at distinct developmental stages or in different tissues and thus, they link ribosome biogenesis to growth and development.

Here, we analyzed the rRNA processing pathway in *Arabidopsis thaliana* by northern blotting of wild type RNA to first define this pathway in more detail. Additionally, to gain further insights into ribosome biogenesis in plants and its link to other processes, we studied two late cofactors in *Arabidopsis thaliana* - the pre-40S endonuclease Nob1 responsible for D-cleavage and the pre-60S GTPase Lsg1 involved in the release of Nmd3 from pre-60S. Both are characterized in unicellular organisms (yeast and archaea), but information for multicellular eukaryotes like plants are lacking. Knock down of the Nob1 ortholog in Arabidopsis (atNob1) results in an accumulation of all precursors leading to 18S rRNA with the strongest accumulation in the P-A₃ fragment. Furthermore, Nob1 links ribosome biogenesis to pollen and embryo development. For Lsg1p we find two orthologs in Arabidopsis (atLsg1-1 and atLsg1-2). Knockout of atLsg1-2 shows significant alterations in the processing pathway. Interestingly, atLsg1-2 links ribosome biogenesis to early leaf development. AtLsg1-1 appears to have lost its ribosome biogenesis function due to the lack of an important basic C-terminus, and we are currently investigating its alternative function.

361-B Towards understanding the inhibitory effects of codon-anticodon wobble base pairing on the ribosome*Hani Zaher*¹, *Rachel Green*²¹Department of Biology, Washington University in St. Louis; ²HHMI, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine

Accurate and rapid translation of the genetic code into functional peptides is a defining feature of the ribosome and the translation factors. The choice of codon within an mRNA has long been known to affect both translational efficiency and accuracy. While the first two positions of the codon are strictly decoded using Watson-Crick base pairing, the third position is often decoded using wobble base pairing. The inosine:adenosine (I:A) wobble base pair, a purine:purine base pair, is perhaps one of the most unusual; for example, the arginine CGA codon is nearly universally decoded by a tRNA isoacceptor bearing an ICG anticodon. In *Saccharomyces cerevisiae*, wobble decoding of the CGA codons appears to have a strong inhibitory effect on translation (Letzrig et al., RNA, 2010). Interestingly, the inhibitory effect is much more pronounced with CGA pairs relative to single codons suggesting synergy between consecutive decoding events. Previously, we have shown that in *E. coli* the quality of the P site tRNA-mRNA interaction has a profound effect on the decoding process in the A site of the ribosome where mismatches in the P site trigger iterated miscoding and eventually lead to premature termination. Given the results in yeast with consecutive CGA codons, we wondered whether the I:A base pair is acting like a mismatch inducing a process similar to that seen with *E. coli*. To this end we constructed reporters bearing stretches of synonymous arginine codons and characterized their translational efficiencies. Similar to earlier findings (Letzrig et al., RNA, 2010), a stretch of four CGA codons was sufficient to inhibit the production of full-length protein product. We further found these truncated products to be stabilized by the addition of the proteasome inhibitor MG132 or in the absence of the ribosome-bound E3 ligase LTN1. Remarkably, similar to the mismatch response seen in *E. coli*, mass-spec analysis of the stabilized truncated products revealed that the C-terminus gets extended with uncoded amino acids following the terminal encoded arginines. These findings suggest that I:A pairing induces iterated miscoding possibly similar to that previously observed in *E. coli*. We are currently extending these studies to identify factors responsible for the recognition of I:A wobble on the ribosome.

Translational Regulation

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 364 B – 397 B

Location: Main Hallway & Sanada Foyer

- 364 B** Translation of the human erythropoietin transcript is regulated by an upstream open reading frame in response to hypoxia
- 367 B** CPEB3-controlled translation regulates memory consolidation
- 370 B** Identification of DDX6 as a cellular modulator of VEGF expression under hypoxia
- 373 B** Homeostatic regulation of AMPA receptors in synaptic plasticity: a posttranscriptional interplay between Caspr1 and the RNA-binding protein ZBP1.
- 376 B** The connection between mRNA binding by the DEAD-box helicase Ded1p and the kinetics of translation initiation.
- 379 B** Structural basis for translation termination by archaeal RF1 and GTP-bound EF1A complex
- 382 B** Human DDX3 interacts with the HIV-1 Tat protein to facilitate viral mRNA translation
- 385 B** Cap-independent translational regulation of mammalian target of rapamycin (mTOR)
- 388 B** Translational regulation of human hemojuvelin expression via upstream open reading frames
- 391 B** Regulation of mRNA translation in late-phase activated macrophages
- 394 B** Regulation of translation of collagen mRNAs by STRAP
- 397 B** Dom34-mediated dissociation of non-translating ribosomes allows efficient restart of translation after stress

364-B Translation of the human erythropoietin transcript is regulated by an upstream open reading frame in response to hypoxia*Cristina Barbosa¹, Luísa Romão¹***¹Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, PORTUGAL**

In mammalian cells, most translational regulation is exerted at the cap-translation initiation step, where the AUG codon is identified and decoded. However, when translation is inhibited by cell stress, alternative mechanisms of translation initiation act to maintain the synthesis of certain proteins required either for the stress response or to aid recovery from the stress stimulus. These pathways are evolutionarily conserved and have been shown to dramatically impact translation. In many cases, features in the 5' untranslated region (UTR) of these mRNAs are important for translational control. These include small structural elements, internal ribosome entry sites and regulatory upstream open reading frames (uORFs). uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF.

In its classical hormonal role, human erythropoietin (EPO) is a glycoprotein synthesized and released mainly from the kidney, which has a key role in hematopoiesis. However, recent studies have revealed that EPO is a multifunctional molecule produced and utilized by many tissues that rapidly responds to different cell stress stimuli and tissue injuries. Thus, it has the potential to be used as a therapeutic target/strategy for the treatment of several human disorders. Understanding the EPO translational control mechanisms will be valuable in the determination of these therapies. Knowing that human EPO transcript presents a 5'UTR with 181 nucleotides containing a 14-codon-uORF, conserved among different species, which might indicate its role in translational regulation, we aimed to prove this hypothesis.

To explore the role of the *EPO* uORF in controlling translation, we cloned the *EPO* 5'UTR, with the intact or disrupted uORF, fused to the firefly luciferase reporter cistron. HepG2, HEK293 and REPC cells were transiently transfected with these constructs. Luciferase activity was measured by luminometry and the corresponding mRNA levels were quantified by real-time RT-PCR to obtain translation efficiencies. Results have shown that the uORF can decrease the main ORF translation efficiency in about 3-fold. In addition, results support the conclusion that reinitiation, and in less extent leaky scanning, are responsible for the main ORF translation. Furthermore, this repression is released under hypoxia, specifically in REPC renal cells, via eIF2a phosphorylation. These findings provide a framework for understanding that production of high levels of EPO induced by hypoxia is also regulated at the translational level.

367-B CPEB3-controlled translation regulates memory consolidation

Hsu-Wen Chao¹, Li-Yun Tsai¹, Yi-Ling Lu¹, Pei-Yi Lin¹, Wen-Hsuan Huang¹, Hsin-Jong Chou¹, Wen-Hsin Lu¹, Ping-Tao Lee¹, Yi-Shuian Huang¹

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Long-term memory requires activity-dependent synthesis of plasticity-related proteins to strengthen synaptic efficacy and consequently consolidate memory. Cytoplasmic polyadenylation element binding protein (CPEB)3 is a sequence-specific RNA binding protein and represses translation via inhibiting the translation elongation factor 2 (eEF2)¹. Activation of N-methyl-D-aspartate (NMDA) receptors in neurons triggers the cleavage of CPEB3 by calpain 2 and subsequently leads to activity-related translation of CPEB3-targeted RNAs². Recent studies indicate that the *Drosophila* homolog of CPEB3, Orb2, of which prion-oligomeric property is required for memory persistence in the fly³ and the glutamine (Q)-rich prion domain in Orb2 can be substituted functionally with the Q-rich motif in CPEB3⁴, raising the question whether mammalian CPEB3 employs prion-like mechanism to sustain long-term memory. To understand the cellular and molecular contributions of CPEB3-controlled translation to learning and memory, we have generated CPEB3 knockout (KO) mice. The null mice display enhanced hippocampus-dependent memory and abnormal synaptic transmission in the Schaffer collateral pathway. Molecular and cellular characterizations reveal that the elevated PSD95 expression results in altered dynamics of NMDA-induced biochemical and morphological changes in the KO neurons. Together, CPEB3 functions as a negative regulator to confine the strength of consolidated memory. Our results overrule the possibility that CPEB3 can function like Orb2 as a prion to promote and persist memory formation.

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370-B Identification of DDX6 as a cellular modulator of VEGF expression under hypoxia

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Vascular endothelial growth factor A (VEGF) is a crucial pro-angiogenic factor, which regulates blood vessel supply under physiologic and pathologic conditions. The VEGF mRNA 5' untranslated region (5'UTR) bears internal ribosome entry sites (IRES), which confer sustained VEGF mRNA translation under hypoxia when 5'cap-dependent mRNA translation is inhibited. VEGF IRES-mediated initiation of translation requires the modulated interaction of *trans*-acting factors. To identify *trans*-acting factors that control VEGF mRNA translation under hypoxic conditions we established an *in vitro* translation system based on human adenocarcinoma cells (MCF-7). Cytoplasmic extracts of MCF-7 cells grown under hypoxia (1% oxygen) recapitulate VEGF IRES-mediated reporter mRNA translation. Employing the VEGF mRNA 5'UTR and 3'UTR in an tobramycin-aptamer RNA affinity purification approach we isolated interacting proteins from translational active MCF-7 extract prepared from cells grown either under normoxia or hypoxia. Interestingly, mass spectrometry analysis identified the DEAD-box RNA helicase 6 (DDX6) that interacts with the VEGF mRNA 5'UTR. Recombinant DDX6 inhibits VEGF IRES mediated translation initiation in normoxic MCF-7 extract *in vitro*. Under hypoxia the level of DDX6 declines and its interaction with VEGF mRNA is diminished *in vivo*. Depletion of DDX6 by RNAi further promotes VEGF expression in MCF-7 cells. Increased secretion of VEGF from DDX6 knock down cells positively affects vascular tube formation of human umbilical vein endothelial cells (HUVEC) *in vitro*. Our results indicate that the decrease of DDX6 under hypoxia contributes to the activation of VEGF expression and promotes its pro-angiogenic function.

373-B Homeostatic regulation of AMPA receptors in synaptic plasticity: a posttranscriptional interplay between Caspr1 and the RNA-binding protein ZBP1.

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11. PDBEB, Doctoral Programme in Experimental Biology and Biomedicine, University of Coimbra, Portugal; 2. Institute for Interdisciplinary Research, University of Coimbra, Portugal; 3. Center for Neurosciences and Cell Biology & Department of Life Sciences, University of Coimbra, Portugal; 23. Center for Neurosciences and Cell Biology & Department of Life Sciences, University of Coimbra, Portugal

Phenomena such as learning and memory rely on mechanisms of synaptic plasticity, which are highly dependent on the modulation of postsynaptic glutamate receptors of the AMPA-type (AMPA receptors). Moreover, a growing body of evidence suggests that posttranscriptional control of AMPAR subunits may also be fundamental for the expression of homeostatic synaptic plasticity, a mechanism known to adjust and stabilize synaptic strength upon chronic changes in neuronal activity. Despite evidence showing mRNA molecules for the GluA1 subunit of AMPARs strategically positioned for local protein synthesis in dendrites, little is yet known about the mechanisms regulating the availability of synaptic AMPAR transcripts and on how they underlie the expression of several forms of plasticity.

Our data identify the transmembrane protein Contactin associated protein 1 (Caspr1) as a novel potential posttranscriptional regulator of the GluA1 subunit of AMPARs. We show that Caspr1 regulates GluA1 protein by increasing GluA1 mRNA levels, an effect dependent on the presence of the 3'untranslated region of the transcript. Moreover, we propose that Caspr1 exerts its effects on GluA1 mRNA levels by activating a signaling cascade downstream of the tyrosine kinase Src, ultimately regulating the phosphorylation status of the Zipcode-binding protein 1 (ZBP1), a well described RNA-binding protein known to regulate the translation of several mRNAs. Furthermore, our results indicate that this mechanism may be regulated by neuronal activity. We demonstrate that a chronic blockade of activity significantly upregulates not only total and surface synaptic levels of GluA1, but also mRNA levels of this subunit. Moreover, protein and mRNA levels of endogenous Caspr1 are also increased upon homeostatic stimuli, as well as levels of phosphorylated ZBP1. Interestingly, it has been described that phosphorylation of ZBP1 disrupts its binding to target mRNAs, allowing the translation of those transcripts. Accordingly, through a ribonucleoprotein immunoprecipitation protocol, we show that although in basal conditions ZBP1 is able to bind to GluA1 mRNA, upon chronic changes in activity levels of ZBP1-bound GluA1 mRNA are significantly decreased.

Taken together, our results start to uncover a novel posttranscriptional mechanism that may be fundamental to regulate synaptic availability of GluA1 transcripts and contribute to the expression of homeostatic plasticity phenomena.

376-B The connection between mRNA binding by the DEAD-box helicase Ded1p and the kinetics of translation initiation.

Ulf-Peter Guenther¹, Frank Tedeschi¹, Akshay Tambe¹, Sarah Geisler¹, Jeff Collier¹, Eckhard Jankowsky¹

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DEAD-box helicases are involved in virtually all steps of the RNA metabolism, but it remains challenging to define the molecular basis of their functions. Here we address this problem for the highly conserved DEAD-box helicase Ded1p from *Saccharomyces cerevisiae*, which functions in translation initiation. Using a modified CLIP assay, we find that Ded1p binds to a large cross-section of cellular mRNAs. Binding sites are found throughout ORFs and on UTRs. Cognate sites do not display apparent sequence signatures. The most pronounced binding sites on the majority of mRNAs are located 30 to 80 nucleotides downstream of the initiation codon, suggesting position-dependent binding pattern of Ded1p on mRNAs. To examine the impact of mRNA binding by Ded1p on protein synthesis rates, we used a temperature-sensitive yeast strain with a genomically encoded Ded1 mutation which only impairs RNA binding, but causes a defect in translation initiation. We quantitatively measured the response of translation initiation to a sudden shift to non-permissive temperature in short time intervals over several minutes and found significant changes in the kinetic profile in the Ded1p mutant, compared to wt Ded1p. This kinetic approach provides a quantitative measure of the effect of Ded1p on translation initiation. We are currently analyzing the kinetics of this process on a transcriptome-wide level, to determine the impact of Ded1p on rate constants of translation initiation for each individual mRNA.

379-B Structural basis for translation termination by archaeal RF1 and GTP-bound EF1A complex*Kan Kobayashi¹, Kazuki Saito², Ryuichiro Ishitani¹, Koichi Ito², Osamu Nureki¹*¹Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan; ²Division of Molecular Biology, Department of Basic Medical Science, The Institute of Medical Science, The University of Tokyo, Japan

Translation termination occurs when one of the three stop codons, UAA, UAG, or UGA, appears at the ribosomal A site. In this process, the stop codons are recognized by the class-I release factors (RFs). The single eukaryotic class-I RF, eRF1, recognizes all three stop codons and catalyzes polypeptide-chain release. The overall shape of eRF1 resembles that of a tRNA molecule. In addition, eRF1 forms a heterodimeric complex with eRF3, an elongation factor 1 α (EF1A)-related essential factor, to complete the overall translation termination process in a GTP-dependent manner. eRF3 is considered to deliver eRF1 to the ribosomal A site in the translation termination process. However, the structural details for the GTPase-mediated decoding of stop codons by eRFs and the detailed molecular mimicry of tRNA remain to be clarified. eRF1 homologues are widely conserved in archaea, suggesting the conservation of a similar translation termination mechanism in archaea. In archaea, archaeal EF1A (aEF1A) functionally interacts not only with tRNA but also archaeal RF1 (aRF1) and aPelota, which facilitates mRNA surveillance, in a GTP dependent manner, and considered to perform three different functions: translation elongation and termination as well as mRNA surveillance. Here, we will report the structure of aRF1 and GTP-bound aEF1A complex determined at 2.3 Å resolution. In the structure, aRF1 interacts with the tRNA recognition site of aEF1A by mimicking the shape of tRNA, and, as a result, the overall structure of the aRF1·aEF1A complex resembles that of the tRNA·EF-Tu (elongation factor from bacteria) complex. This strongly supports our proposal that aEF1A delivers aRF1 to the ribosomal A site in the translation termination as well as tRNA during the translation elongation. Furthermore, we will describe structural basis for the translation termination process in archaea.

382-B Human DDX3 interacts with the HIV-1 Tat protein to facilitate viral mRNA translation*Ming-Chih Lai¹, Shaw-Jenq Tsai¹, H. Sunny Sun¹*¹**National Cheng Kung University**

Nuclear export and translation of intron-containing viral mRNAs are required for HIV-1 gene expression and replication. In this report, we provide evidence to show that DDX3 regulates the translation of HIV-1 mRNAs. We found that knockdown of DDX3 expression effectively inhibited HIV-1 production. Translation of HIV-1 early regulatory proteins, Tat and Rev, was impaired in DDX3-depleted cells. All HIV-1 transcripts share a highly structured 5' untranslated region (UTR) with inhibitory elements on translation of viral mRNAs, yet DDX3 promoted translation of reporter mRNAs containing the HIV-1 5' UTR, especially with the transactivation response (TAR) hairpin. Interestingly, DDX3 directly interacts with HIV-1 Tat, a well-characterized transcriptional activator bound to the TAR hairpin. HIV-1 Tat is partially targeted to cytoplasmic stress granules upon DDX3 overexpression or cell stress conditions, suggesting a potential role of Tat/DDX3 complex in translation. We further demonstrated that HIV-1 Tat remains associated with translating mRNAs and facilitates translation of mRNAs containing the HIV-1 5' UTR. Taken together, these findings indicate that DDX3 is recruited to the TAR hairpin by interaction with viral Tat to facilitate HIV-1 mRNA translation.

385-B Cap-independent translational regulation of mammalian target of rapamycin (mTOR)*Ana Marques-Ramos¹, Alexandre Teixeira², Rafaela Lacerda¹, Lúisa Romão¹***¹Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal and Universidade de Lisboa, Faculdade de Ciências, Center for Biodiversity, Functional and Integrative Genomics, Lisboa, Portugal;****²Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal and Centro de Investigação em Genética Molecular Humana, Faculdade de Ciências Médicas, Universidade Nova de Lisboa, Portugal**

Regulation of translation is a key mechanism by which cells and organisms can rapidly change their gene expression patterns in response to extra- and intracellular stimuli. Translational control can occur on a global basis by modifications of the basic translation machinery, or selectively target defined subsets of mRNAs to maintain the synthesis of certain proteins required either for the stress response or to aid recovery from the stress. These pathways are evolutionary conserved and have been shown to significantly impact translation in organisms as diverse as yeast and humans. In many cases, features in the 5' untranslated regions (5'UTRs) of the corresponding mRNAs, such as regulatory upstream open reading frames (uORFs) and internal ribosome entry sites (IRESs) are important for them to evade global repression of translation. IRES-mediated translation is an alternative to the cap-dependent mechanism of translation initiation that involves the direct recruitment of the ribosome to the vicinity of the initiation codon and may require several trans-acting proteins known as IRES trans-acting factors (ITAFs).

Mammalian target of rapamycin (mTOR) is a highly conserved kinase that is responsive to several cellular stimuli. Deregulation of mTOR signalling is implicated in major diseases, such as cancer, mainly due to its role in regulating protein synthesis. The main mTOR targets are proteins responsible for ribosome recruitment to the mRNA, thus, a specific inhibitor of mTOR, for example rapamycin, leads to global inhibition of translation. Knowing that in stress conditions such as hypoxia, overall protein synthesis is reduced, but synthesis of mTOR protein is not inhibited, we hypothesized that mTOR 5'UTR harbours an IRES allowing cap-independent synthesis of mTOR protein in stress conditions. By using dicistronic reporter plasmids we have tested and confirmed this hypothesis. Our findings provide a framework for understanding how translational reprogramming of mTOR mRNA is regulated in response to cellular stress conditions.

388-B Translational regulation of human hemojuvelin expression via upstream open reading frames*Cláudia Onofre¹, Cristina Barbosa¹, Luísa Romão¹***¹Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, PORTUGAL**

Hemojuvelin (HJV) is a glycosylphosphatidylinositol (GPI)-linked membrane protein shown to be a co-receptor for class of ligands called bone morphogenetic proteins (BMPs). The HJV is involved on iron homeostasis through regulation of hepcidin transcription levels. Mutations on the hepcidin gene or in the HJV gene cause an early-onset inherited disorder associated to iron overload named juvenile hemochromatosis.

A better knowledge of the mechanisms implicated in HJV gene expression is crucial to understand its role in the iron homeostasis. The 5' leader sequence of the human HJV mRNA has two upstream open reading frames (uORF) with 28 and 19 codons created by two upstream AUGs (uAUGs) that share the same stop codon. Reporter constructs containing several HJV 5' leader sequences fused to the Firefly luciferase cistron were tested in HeLa and HepG2 cells to evaluate the effect of these uORFs in the translational regulation of HJV mRNA. Luciferase activity was measured by luminometry and the corresponding mRNA levels, quantified by real-time RT-PCR.

The results revealed that the HJV uORFs decrease the translational efficiency of the main ORF in about 6-fold. Furthermore, we have observed that the production of HJV protein is mainly due to translation reinitiation. Thus, HJV mRNA has a low leaky scanning ability that contributes to the translational repression of the main ORF. We also observed that the amino acid sequence of the uORF2-encoded peptide seems to cause ribosomal stalling, which also impedes translation of the downstream main ORF. In addition, our preliminary results show that in HepG2 cells submitted to eIF2a phosphorylation or iron overload, the HJV uORFs-mediated translational repression is released. These results suggest that these uORFs play a role in regulating HJV expression levels in response to iron overload.

391-B Regulation of mRNA translation in late-phase activated macrophages*Sonja Reitter¹, Johanna Schott², Georg Stoecklin²*¹German Cancer Research Center, DKFZ-ZMBH Alliance, Heidelberg, Germany; ²German Cancer Research Center, DKFZ-ZMBH Alliance, Heidelberg, Germany

Macrophages strongly respond to different inflammatory stimuli such as bacterial lipopolysaccharide (LPS) with a coordinated release of cytokines, the production of oxygen radicals and by stimulating phagocytosis. In the early phase of activation, macrophages primarily secrete pro-inflammatory cytokines that trigger the immune response. Late after activation, macrophages produce anti-inflammatory cytokines to dampen the immune reaction and resolve inflammation. Moreover, macrophages in the late phase enter a state of unresponsiveness (endotoxin tolerance), during which they cannot be re-activated. This sequence of events requires tightly coordinated changes in the gene expression program of macrophages. While the regulation of transcription and mRNA stability during macrophage activation has been studied extensively, much less is known about the regulation of translation. Here we present a systematic analysis of changes in mRNA translation that occur in late-phase activated macrophages.

By recording polysome profiles of mouse RAW264.7 macrophages, we observed a global suppression of mRNA translation in late-phase activated macrophages. The percentage of polysomal ribosomes was reduced by 50% after 16 hours of LPS treatment. To identify mRNAs whose translation rate is regulated specifically during this response, we performed microarray analysis of mRNAs associated with light and heavy polysomes. Thereby, we discovered 61 messenger RNAs that escape global translation suppression, indicating that their protein products are important for the late phase of macrophage activation. These mRNAs encode inhibitors of the NFκB transcription factor complex, proteins involved in producing reactive oxygen and nitrogen species, and a family of small GTPases known to participate in microbial defense. In addition, our analysis identified 52 mRNAs whose translation is actively suppressed in late-phase activated macrophages, including mRNAs encoding transcription factors and activators of inflammation. Our results strongly suggest that translation control contributes to the unresponsive state of late-phase activated macrophages. Currently, we are in the process of identifying regulatory elements within a selected number of the identified mRNAs. This will serve as a basis for further investigating the mechanisms by which specific control of translation is achieved.

394-B Regulation of translation of collagen mRNAs by STRAP*Lela Stefanovic¹, Milica Vukmirovic¹, Branko Stefanovic¹*¹College of medicine, florida State University

Type I collagen is the most abundant protein in human body and is composed of two $\alpha 1(I)$ and one $\alpha 2(I)$ polypeptides, which are encoded by two different mRNAs. However, both collagen mRNAs contain in their 5'UTR a conserved structure, termed the 5' stem-loop (5'SL). We have cloned LARP6 as the protein which binds 5'SL with high affinity and specificity. This binding regulates translation of collagen mRNAs. Among other interactions, LARP6 also interacts with protein STRAP (also called unrip). STRAP is involved in translation of IRES containing mRNAs by interacting with protein unr and in formation of snRNPs by interacting with Gemin 7. The last 20 amino-acids at the C-terminus of LARP6 are required for its interaction with STRAP, these amino-acids are conserved in unr and Gemin 7, representing the STRAP interacting epitope. Overexpression of STRAP restricts translation of collagen mRNAs, as evidenced by lower protein level and shift of collagen mRNAs from heavy polysomes. Knock out of STRAP results in overexpression of collagen $\alpha 1(I)$ and $\alpha 2(I)$ polypeptides and high loading of collagen mRNAs onto polysomes. However, collagen polypeptides in the STRAP knock out cells are hyperglycosylated and fail to assemble into collagen triple helix. The hyperglycosylation takes place if the synthesis of $\alpha 1(I)$ and $\alpha 2(I)$ polypeptides is not coordinated, the randomly made polypeptides fail to productively fold into triple helix and are exposed to the modifying enzymes for prolonged period of time. These results suggest that STRAP restricts random translation of collagen mRNAs and that this inhibition is necessary to coordinate translation of collagen $\alpha 1(I)$ mRNA to that of $\alpha 2(I)$ mRNA. A working model of regulation of collagen translation by STRAP will be presented.

397-B Dom34-mediated dissociation of non-translating ribosomes allows efficient restart of translation after stress*Antonia van den Elzen¹, Bertrand Séraphin¹***¹Equipe Labellisée La Ligue, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de Recherche Scientifique (CNRS) UMR 7104/Institut National de Santé et de Recherche Médicale (INSERM) U964/ Université de Strasbourg, 67404 Illkirch, France**

Dissociation of terminating ribosomes ensures the availability of the resulting subunits for new rounds of translation. Upon glucose depletion in yeast, translation rapidly ceases and free ribosomal subunits tightly associate, tethered by the Stm1 factor, forming a large pool of non-translating ribosomes. A mechanism dissociating Stm1-bound ribosome and allowing the reentry of these subunits in translation is thus required upon glucose repletion. Similar mechanisms must also exist in all cells as non-translating ribosomes temporarily accumulate under a variety of stress conditions and need to be released when translation resume.

The Dom34 and Hbs1 factors form a complex structurally similar to the one resulting from association of translation termination factors eRF1 and eRF3. Dom34 and Hbs1 were initially characterized as implicated in the NGD RNA Quality Control Pathway. Consistently, recent biochemical analyses demonstrated that Dom34 and Hbs1, together with the factor Rli1, dissociated ribosomes stalled on a messenger RNA. These factors were also shown to dissociate associated ribosomal subunits *in vitro*. Dom34 and Hbs1 are not essential for viability in yeast and the extent of their physiological function(s) remains unclear. Using a variety of assays, we found that Dom34-Hbs1 mediates the dissociation of non-translating ribosomes present in glucose-starved yeast, facilitating thereby an efficient restart of translation when glucose is supplied again. Our data show for the first time that Dom34 and Hbs1 dissociate non-stalled, mature ribosomes *in vivo*. This suggests that these factors might have a general physiological role in affecting ribosomal subunit availability, thus impacting on translation initiation, possibly even in non-stress conditions.

3' end processing

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 400 B – 412 B

Location: Main Hallway & Sanada Foyer

400 B **Alternative polyadenylation in CD2 expression**

403 B **Role of mRNA 3' processing in the progression of the DNA damage response (DDR)**

406 B **Towards the understanding of the CTD-code**

409 B **Mammalian mRNA 3'end formation: is this the end? New insight on CstF64**

412 B **ENHANCER OF RNA INTERFERENCE -1-LIKE-1: THE ERI1 PLANT HOMOLOGUE INVOLVED IN THE RNA PROCESSING GAME OF THE CHLOROPLAST**

400-B Alternative polyadenylation in CD2 expression

Inês Boal-Carvalho¹, Mafalda Pinto³, Juliana Miranda², João Relvas⁴, Teresa Summavielle⁵, Alexandre Carmo³, Alexandra Moreira¹

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Alternative polyadenylation (APA) in the 3'UTR has a fundamental role in gene expression in a variety of cellular programs: cellular differentiation, leukocyte activation and cancer. The physiological relevance of APA was accentuated by the observation that human T lymphocytes stimulated through T cell receptors express preferentially mRNA isoforms with shorter 3'UTRs. CD2 is a T cell surface glycoprotein involved in signaling transduction and T cell activation. Transcription of CD2 results in the formation of multiple mRNA species whose physiological function has remained undisclosed. CD2 contains a conserved 3'UTR across species, from rodents to humans, which may indicate the presence of regulatory elements in this region. CD2 3'UTR contains a proximal and a distal poly(A) signals that are used in human T lymphocytes to generate two mRNA species with different 3'UTR. In mouse, however, only the shorter mRNA produced by usage of the first poly (A) site has been reported in T cells. By immunohistochemistry, Northern blotting and RT-PCR using perfused rat brains, we now present evidence that CD2 is also expressed in different regions of the brain, specifically in the cortex, cerebellum, hippocampus and thalamus, which represent a significant part of the encephalon. CD2 appears to have a very low expression in the hippocampus, contrary to what happens in the cortex where relatively high levels of expression were detected. Additionally, we have mapped the 3' ends of the CD2 mRNA isoforms by 3'RACE/sequencing to show that the two poly(A) signals are used generating two mRNAs with different 3'UTR lengths, both in the Jurkat T cell line as well as in the rat brain. Although some reports have described the expression of immune molecules in neurons the role of these molecules in the nervous system is still unknown. As the nervous system is similar to the immune system with respect to many phenomenological, functional and molecular properties, a potential role for CD2 in the brain will be discussed.

This work is funded by FEDER through Programa Operacional Fatores de Competitividade – COMPETE – and by National Funds through FCT - Fundação para a Ciência e a Tecnologia – project ref: PTDC/SAU-GMG/116621/2010 and FCOMP-01-0124-FEDER-022718 (PEst-C/SAU/LA0002/2011).

403-B Role of mRNA 3' processing in the progression of the DNA damage response (DDR)*Emral Devany*¹, *Xiaokan Zhang*², *Mirjana Persaud*², *Frida Kleiman*²¹Chemistry and Biochemistry Dept., Hunter College and City University of New York; ²Chemistry and Biochemistry Department, Hunter College and Graduate Center of City University of New York

The cellular response to DNA damage could be either in the survival mode, where DNA repair and cell cycle arrest occur, or in the death mode, where apoptosis is induced. The mRNA 3'-end processing machinery is involved in the response to DNA damage. As the poly(A) tail in the 3' end of mRNAs is important to control mRNA stability, mRNA export and translation; the regulation of 3'-end processing during DDR represents an important mechanism to control gene expression. Recently, we have described a novel feedback loop between p53 and polyA specific ribonuclease (PARN), where PARN deadenylase keeps p53 levels low in non-stress conditions by destabilizing p53 mRNA through the AU-rich elements (AREs) present in the 3'UTR. After UV, the p53 expression levels increase resulting in the p53-mediated activation of PARN deadenylase, which represents a mechanism of p53-mediated regulation of gene expression in a transactivation-independent manner.

As the levels of p53 expression increase after DNA damage treatment, the PARN-mediated down-regulation of p53 mRNA should be reverted during the progression of DDR. It has been shown that HuR, an ubiquitously expressed ARE-binding protein, binds the AREs in p53 3'UTR resulting in the regulation of p53 mRNA translation and expression upon stress. Our results also indicate that under normal conditions Ago2 and PARN bind to a region in the p53 3'UTR where a miRNA-binding site and ARE sequence overlap, and both of them dissociate after UV treatment. Interestingly, our results indicate that under DNA damaging conditions HuR can compete for binding to the p53 3'UTR with both PARN and Ago2 in *in vitro* and *in vivo* assays, resulting in the release of PARN and Ago2 and in the increase of p53 expression levels. Based on these results, our working model is that the dynamic binding of PARN, Ago2 and HuR to the p53 3'UTR plays a role in the progression of DDR.

Together our studies provide new insights into p53 function and the mechanisms behind the regulation of mRNA 3' end processing in different cellular conditions, providing new approaches in the design of new cancer therapies.

406-B Towards the understanding of the CTD-code

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RNA polymerase II (RNAPII) is not only the fundamental enzyme involved in the gene expression but also the central coordinator of co-transcriptional processing. RNAPII associates with a large number of enzymes and protein/RNA-binding factors through its C-terminal domain (CTD). The CTD consists of tandem repeats of the heptapeptide consensus Y1S2P3T4S5P6S7. The CTD is dynamically post-translationally modified, e.g. phosphorylated at various positions. These phosphorylations creates specific patterns of the CTD, which predominate at different stage of the transcription cycle, control the recruitment, activation, and displacement of various factors involved in transcription and RNA processing.

The CTD is flexible but it can acquire diverse structures upon binding with corresponding protein factors. Our study that combines various approaches ranging from nuclear magnetic resonance (NMR), through fluorescent anisotropy (FA) to small angle x-ray scattering (SAXS) aims to understand how the full-length CTD is modulated upon binding with multiple copies of protein factors containing CTD-interacting domains. By combining the recombinant protein technology, with the afore mentioned techniques we are trying to uncover how proteins are organized along the whole CTD length at atomic level. Revealing the structural behavior of the CTD will help to understand the role of CTD and its post-translational modifications in the regulation of the transcription cycle.

409-B Mammalian mRNA 3'end formation: is this the end? New insight on CstF64*Valentina Romeo*¹, *Daniel Schümperli*²¹Institute for Cell Biology, University of Bern; ²University of Bern, Institute for Cell Biology

Constitutive polyadenylation (CPA) and alternative polyA site selection (APS) participate in the control of cell life. Together with splicing, they generate alternative mRNA/protein isoforms, and/or mRNAs containing different regulatory elements in their 3' untranslated regions. Alterations of these reactions often occurs in cancer and in neurological disorders. In this context CstF plays a critical role. This complex consists of three subunits of 77, 64 and 50 kDa and seems to act as a hexamer at least in some steps of CPA. CstF64 binds preferentially to a G/U rich downstream sequence element and thereby helps to define the polyadenylation site. Several reports indicate that CstF64 is crucial in cell growth and development. Importantly, several tissue-specific splicing isoforms of CstF64 and a paralogue on chromosome 19 (CstF64Tau) have been described. This leads to our main hypothesis that changes in CstF64/CstF64Tau ratio or in the abundance of various CstF64 splicing isoforms may be important in regulating APS in cell growth and development. Interestingly, CstF77 and 64, but not 50, are also involved in replication-dependent histone RNA 3' end processing even if there is no direct evidence of any RNA binding activity. This obviously raises questions about the function of CstF in histone mRNA processing.

Firstly, we analysed the CstF64/CstF64Tau ratio in HeLa cells throughout the cell cycle. This revealed a small but interesting differential expression of the two paralogues in diverse phases. Moreover, by using an inducible knock-down system, we were able to show a defect of CstF64 (and for less extends CstF64T) lacking cells in G1 to S phase transition. Our current research aims to shed light on the role of CstF64 and CstF64Tau in cell cycle progression and/or histone mRNA 3'end processing. We are currently performing IPs, in vivo processing assay for histone mRNAs and dissecting the molecular basis of this deregulation by studying recombinant protein interactions.

Secondly, we investigated the CstF64 splicing pattern during retinoic acid-induced differentiation of the human neuroblastoma cell line SH-SY5Y. In this system we detected a concrete and substantial change in the ratio of specific splicing isoforms. Interestingly, the protein variants differ in their predicted structures and possibly protein:protein interaction properties. We are characterizing the biological implication of these changes by knocking down specifically all the endogenous CstF64 isoforms and expressing RNAi-resistant versions of individual variants. In such context, we plan to study global polyadenylation site preferences by polyA-seq experiments and proteins associated with the expressed CstF64 variants by immunoprecipitation. Furthermore, we will analyse the functional impact of these CstF64 isoform manipulations on cell differentiation and growth.

412-B ENHANCER OF RNA INTERFERENCE -1-LIKE-1: THE ERL1 PLANT HOMOLOGUE INVOLVED IN THE RNA PROCESSING GAME OF THE CHLOROPLAST

*Ioannis Vlatakis*¹, *Glykeria Mermigka*², *Jutta Helm*², *Heiko Schumacher*², *Eugenia Vamvaka*², *Kriton Kalantidis*¹

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Ribonucleases are a group of enzymes widely distributed in nature. In the chloroplasts, a network of post-transcriptional modifications of RNA molecules, is mediated by ribonucleases. Our lab found that ENHANCER OF RNA INTERFERENCE-1-LIKE-1 (ERL1, named following plant nomenclature conventions) in *Arabidopsis thaliana*, is such an enzyme. ERL1 belongs to a family of exoribonucleases which share a common 3'-5' exonuclease domain (EXOIII domain) containing a highly conserved DEDD motif. Homologues of ERL1 fulfill various functions in RNA metabolism by participating in tRNA and rRNA processing in bacteria and in the regulation of RNAi and rRNA maturation pathways in eukaryotes. By confocal microscopy we showed that *Arabidopsis* ERL1 is targeted to the chloroplasts. We have generated *Arabidopsis thaliana* and *Nicotiana benthamiana* plants that misexpress ERL1. The misexpression of ERL1 leads to phenotypes indicative of defects in chloroplasts development in both plant species. Since ERL1 acts in the chloroplast, we used these transgenic lines to study the impact of ERL1 on chloroplastic related genes and measured the chlorophyll content. In addition we showed that in vitro purified ERL1 protein is capable of processing different RNA substrates (5S rRNA precursor and siRNA-like oligos). Recently, an RNA sequencing analysis complemented by Northern blot analysis has showed alterations at the expression and processing levels of certain operons in the absence of plant ERL1. Altogether our results suggest that ERL1 is another piece in the puzzle of the complex posttranscriptional regulatory machinery of the chloroplasts.

RNA Turnover

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 415 B – 445 B

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- 415 B** Regulation of stress granules formation during calicivirus infection
- 418 B** mRNA degradation on the ribosome in *Drosophila* cells
- 421 B** Control of mRNA metabolism by deadenylation
- 424 B** Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in human, fish, and fly
- 427 B** DHX34 activates NMD by promoting the transition from the SURF to the DECID complex.
- 430 B** Investigation of premature termination codon recognition in nonsense-mediated mRNA decay
- 433 B** Direct Visualization of Alternative RNA Substrate Recruiting Pathways in Yeast Exosome
- 436 B** Functional analysis of IMP3, a RNA-binding protein
- 439 B** Assessing differential susceptibility of mRNAs to NMD
- 442 B** Mpn1, mutated in poikiloderma with neutropenia protein 1, unveils cellular surveillance of catalytic spliceosomal small nuclear RNAs.
- 445 B** CBP1 mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner

415-B Regulation of stress granules formation during calicivirus infection

MAJID AL-SAILAWI¹, Nicolas Locker¹, Elizabeth Royall¹, Lisa Roberts¹

¹University of Surrey

Caliciviruses are single-stranded positive RNA viruses that are responsible for several important diseases in human and animal hosts. To date, the replication mechanisms of human caliciviruses are poorly understood because of a lack of a suitable cell culture system. Feline calicivirus (FCV) or mouse norovirus (MNV) share many properties with the human caliciviruses, and provide models to increase our understanding of calicivirus translation and replication.

Recent evidence suggests that the dynamic nature of mRNA expression is a key coordinator of viral pathogenesis, with different host genes expressed at different times during infection. The expression of mRNAs can be regulated through their storage and/or decay in subcellular compartments such as stress granules, to stall their translation, or processing bodies (P-bodies), for their further degradation. Moreover, proteins within P-bodies or stress granules can enhance or limit viral infection. Viral proteins can also be found in these compartments, suggesting an important interplay between RNA turnover and viral life cycle.

This is an exciting emerging field in virology and we have set out to investigate how calicivirus infection affects and regulates the formation of P-bodies and stress granules for efficient replication and will present evidence that calicivirus infection modulates the formation of stress granules.

418-B mRNA degradation on the ribosome in *Drosophila* cells*Sanja Antic*¹, *Anna Skucha*¹, *Silke Dorner*¹¹Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

The translation and degradation of mRNAs are two key steps in gene expression. Consequently, both processes are highly regulated and targeted by many factors including miRNAs. Even though translation and mRNA degradation are tightly coupled, it was suggested that mRNAs are degraded after their dissociation from the ribosomes in cytoplasmic bodies, named P-bodies. Only recently the possibility of co-translational mRNA degradation in yeast was discussed. However, at this point it is unclear whether mRNA degradation on the ribosome would be limited to particular mRNA species and could also occur in multicellular eukaryotes.

We have investigated the possibility of co-translational mRNA degradation in *Drosophila* cells. The co-purification of mRNA degradation factors with heavy fractions of polysome profiles of cell lysates was an important first step. However, since P-bodies could co-migrate with heavy polysome fractions we further established the affinity purification of ribosomes from *Drosophila* S2 cell lysates. We could demonstrate the co-purification of various deadenylation and decapping factors with ribosomes. Interestingly, also the factors of the miRNA effector components, AGO1 and GW182, co-purify with ribosomes. Ongoing experiments investigate the general abundance of decapped mRNAs on ribosomes. In summary our findings strongly suggest the ribosome as an alternative site for mRNA degradation in *Drosophila*.

421-B Control of mRNA metabolism by deadenylation*Jeff Collier¹*¹Case Western Reserve University

The presence of a polyadenosine tail is an important determinant of mRNA translation and stability. The regulated removal of the tail, i.e. deadenylation, leads to either translational quiescence or mRNA degradation. Deadenylation, therefore, is a critical node in gene expression and is especially important in certain biological contexts such as in the early embryo and in neurons. All mRNAs deadenylate at different rates; some fast, some slow. In addition, deadenylation is enhanced by message-specific regulatory factors. For instance, many 3' UTR binding proteins and the miRNA machinery drive post-transcriptional regulation (in part) by facilitating deadenylation. Despite the importance of deadenylation, little is known about how differential rates of poly(A) tail shortening are achieved. One clue comes from studies that show that mRNA translation rates are intimately connected to deadenylation rate. Our recent work has begun to tease out the complex molecular details that intertwine translation and deadenylation. Moreover, we have concentrated on understanding the function of protein factors that facilitate removal of the poly(A) tail. Deadenylation is catalyzed by the CCR4-NOT protein complex. The function of distinct members of this complex as well as how these factors influence rates of deadenylation will be discussed.

424-B Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in human, fish, and fly

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¹University of California, Berkeley; ²Broad Institute of MIT and Harvard; ³Chinese Academy of Sciences; ⁴Fudan University; ⁵Ohio State University; ⁶University of Connecticut

Many alternatively spliced isoforms contain a premature termination codon that targets them for degradation by the nonsense-mediated mRNA decay RNA surveillance system (NMD). Some such unproductive splicing events have a regulatory function, whereby alternative splicing and NMD act together to impact protein expression. Numerous RNA-binding proteins, including all the human SR splicing factors, are regulated by alternative splicing coupled to NMD, in conjunction with highly- or ultra-conserved elements [1,2]. The "50nt rule" is the prevailing model for how premature termination codons are defined in mammals, and requires a splice junction downstream of the stop codon. [3]. There is evidence that this rule holds in *Arabidopsis* [4] but not in other eukaryotes including *Drosophila* [5]. There is also evidence that a longer 3' UTR triggers NMD in yeast, plants, flies, and mammals [4,6,7].

To survey the targets of NMD genome-wide in human, zebrafish, and fly, we have performed RNA-Seq analysis on cells where NMD has been inhibited via knockdown of UPF1, a critical protein in the degradation pathway. We found that hundreds to thousands of genes produce alternative isoforms that are degraded by NMD in each of the three species, including over 20% of the genes alternatively spliced in human HeLa cells. These genes, potentially subject to regulation through NMD, are involved in many functional categories and, in human and fly, are significantly enriched for RNA splice factors, indicating that auto- and cross-regulation of splice factors through NMD is widespread. We also found a significant enrichment for ultraconserved elements in the human NMD targets, and usually these elements overlapped a poison cassette exon.

We were able to gain insight into what defines NMD targets from our RNA-Seq data. We found that the 50nt rule is a strong predictor of NMD degradation in human cells, and also seems to play a role in zebrafish and, surprisingly, in fly. In contrast, we found little correlation between the likelihood of degradation by NMD and 3' UTR length in any of the three species. In fly, we see no enrichment for longer 3' UTRs in isoforms degraded by NMD, unless they have an intron. Other features have also been associated with propensity for NMD. We also found that thousands of transcripts have uORFs that seem to affect their likelihood of degradation.

Ultimately, our findings demonstrate that gene expression regulation through NMD is widespread in human, zebrafish, and fly, and that NMD is strongly predicted by the 50nt rule but not by 3' UTR length.

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427-B DHX34 activates NMD by promoting the transition from the SURF to the DECID complex.*Nele Hug*¹, *Oscar Llorca*², *Javier Cáceres*¹¹MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, UK; ²Spanish National Research Council (CSIC), Centro de Investigaciones Biológicas (CIB), Ramiro de Maeztu 9 Madrid (Spain)

Nonsense-mediated decay (NMD) is a surveillance mechanism that degrades aberrant mRNAs. A complex comprising the NMD factors SMG1 and UPF1 and the translation termination factors eRF1 and eRF3 (SURF) is assembled in the vicinity of a premature termination codon. Subsequently, an interaction with the exon junction complex induces the formation of the decay-inducing complex (DECID) and triggers NMD (1). We previously identified the DExD/H box protein DHX34 as a novel NMD factor in *C. elegans* and showed that it acts in the NMD pathway both in human cells and also in zebrafish (2,3). Here, we investigate the mechanism by which DHX34 activates NMD in human cells. We show that DHX34 is an RNA-binding protein that is recruited to the SURF complex via its preferential interaction with hypo-phosphorylated UPF1 and the kinase SMG-1. A series of molecular transitions induced by DHX34 include an enhanced recruitment of UPF2, increased UPF1 phosphorylation and finally dissociation of eRF3 from UPF1. These molecular transitions are dependent on the ATPase activity of DHX34. Altogether, these results show that DHX34 has a central role in NMD by triggering the conversion from the SURF into the DECID complex. Undergoing structural studies using cryo-EM with DHX34 in combination with NMD core factors will help establish the precise mechanism of DHX34 in the activation of NMD.

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430-B Investigation of premature termination codon recognition in nonsense-mediated mRNA decay*Raphael Joncourt¹, Andrea Eberle², Oliver Mühlemann¹***¹Department for Chemistry and Biochemistry, University of Bern, Switzerland; ²Department of Molecular Biology and Functional Genomics, Stockholm University, Sweden**

Nonsense-mediated mRNA decay (NMD) is best known for its role in quality control of mRNAs. Premature translation termination codons (PTCs) are recognized and the corresponding mRNA gets rapidly degraded. The basic mechanism of NMD appears to be conserved from yeast to mammals. According to the current working model, aberrant translation termination leads to NMD. It is thought that correct termination requires the interaction of the ribosome at the stop codon with the poly(A)-binding protein (PABP) mediated through eukaryotic release factor 3 (eRF3). The model predicts that in the absence of this interaction, the NMD core factor UPF1 binds to eRF3 instead and initiates the assembly of an NMD complex that ultimately leads to mRNA degradation. However, the exact mechanism that allows distinguishing between proper and aberrant (i.e. NMD-inducing) translation termination is not yet well understood.

We address this question using a tethering approach in which proteins of interest are bound to a reporter transcript into the vicinity of a PTC. Subsequently, the ability of the tethered proteins to inhibit NMD and thus to stabilize the reporter transcript is assessed. Preliminary results revealed that the C-terminal domain interacting with eRF3 seems not to be necessary for tethered PABP to suppress NMD. In contrast, the N-terminal part of PABP, consisting of 4 RNA recognition motifs (RRMs) and interacting with eukaryotic initiation factor 4G (eIF4G), retains the ability to inhibit NMD. Tethered eIF4G is also able to stabilize the reporter transcript, it is however not yet clear if this is a direct or indirect effect. There are indications that this stabilization might be due to endogenous PABP being recruited, but also that the interaction of eIF4G to initiation factor 3 (eIF3) might play a role. The results obtained so far suggest that PABP undoubtedly has an important function for the suppression of NMD, but that there might be other factors able to complement or circumvent its actions.

433-B Direct Visualization of Alternative RNA Substrate Recruiting Pathways in Yeast Exosome*Jun-Jie Liu¹, Ailong Ke², Hong-Wei Wang¹*¹School of Life Sciences, Tsinghua University; ²Cornell University

The multi-subunit exosome complex plays crucial roles in RNA degradation and processing both in cytoplasm and nucleus by cleaving RNA substrates from their 3' end. From archaea to eukaryotes, exosome has a structurally conserved core complex consisting of nine subunits forming a ring-like structure with a channel in the center allowing single stranded RNA to go through. Eukaryotic core complex has lost its exonuclease activity but gains its RNase activity through two additional hydrolytic 3' to 5' exoribonucleases, Rrp44 and Rrp6, that both bind to the core. Rrp44 degrades RNA substrates processively while Rrp6 degrades RNA distributively.

Structural analysis revealed that Rrp44 protein situates at the bottom of the core facing the core channel exit. In tune with a similar function of the archaeal exosome, biochemical data indicate that RNA substrates with long single stranded 3' overhangs are first channeled through the eukaryotic exosome core before being degraded by Rrp44. This "through-core" route is supported by the most recent crystal structure of the yeast Rrp44-exosome (RE) in complex with an RNA substrate with a 5' hairpin and long 3' end single stranded overhang. On the other hand, recent transcriptom data suggested the presence of alternative route for RNA substrates with shorter 3' end single stranded overhangs to be processed by exosome. Our previous reconstruction of the apo-RE complex revealed multi-porous structure suggesting the potential for RNA substrates to take multiple routes including the "through-core" and some "direct access" routes to get access to Rrp44's exonuclease site.

In order to further probe the mechanism of RNA degradation by exosome especially the recruitment route of RNA substrates, we have performed biochemical and single particle electron microscopy analysis on yeast Rrp44-exosome in concert with RNA substrates. We used model structured RNA and linear RNA substrates with 3' end single stranded tails of various lengths as a molecular ruler to determine the length of RNA required for interaction with and degradation by the exosome. We exploited single particle reconstruction of the RE-RNA complexes to reveal the substrate induced conformational change of the complex. Single particle electron microscopy analysis of Rrp44-exosome in complex streptavidin-labeled RNA substrates provided us direct visual evidence of the alternative RNA recruiting pathways by Rrp44-exosome complex.

436-B Functional analysis of IMP3, a RNA-binding protein*Rena Mizutani*¹, *Yutaka Suzuki*², *Nobuyoshi Akimitsu*¹¹Radioisotope Center, The University of Tokyo, Tokyo, Japan; ²Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan

RNA-binding proteins regulate multiple steps of post-transcriptional gene expression, and it is important to know the target RNAs of RNA-binding proteins. The RNA binding protein IMP3 (Insulin-like growth factor 2 (IGF-2) mRNA-binding protein 3) is an oncofetal protein, emerging as a useful indicator of the progression and prognoses of several cancers. According to preceding study, IMP3 binds to IGF-2 mRNA thereby activating its translation, and stabilizes CD44 mRNA. IMP3 has a potential not to be simply a marker of prognosis in cancer cells, but also to regulate cancer progression. However, little is known about the function of IMP3 in cancer cells because transcripts regulated by IMP3 are unclear. In this study, we tried to identify RNA targets of IMP3 through genome-wide analysis.

Initially, we tried to identify RNAs bound to IMP3 by RNA immunoprecipitation followed by deep sequencing (RIP-seq). We then determined 2201 RNAs enriched in IMP3 immunoprecipitant as candidates of IMP3 binding RNAs. We also determined the expression levels of whole transcripts in IMP3-depleted HeLa TO cells by deep sequencing analysis. The expression levels of 65 transcripts were increased in IMP3-depleted cells. Among them, 15 transcripts were bound to IMP3, and we judged these 15 transcripts are regulated by IMP3. We measured the stability of the RNA targets of IMP3 in IMP3-depleted HeLa TO cells using BRIC-method, an inhibitor-free method for directly measuring RNA stability. CDK18 mRNA, SOCS2 mRNA, LRFN3 mRNA and CCDC92 mRNA were stabilized by depletion of IMP3. Our results suggest that IMP3 promotes RNA degradation of RNA targets, and might regulate cell growth through control of RNA stability of *CDK18* and *SOCS2*, negative regulators of cell growth. This is a first demonstration that IMP3 is a RNA destabilizing factor for a set of transcripts.

439-B Assessing differential susceptibility of mRNAs to NMD*Christoph Schweingruber*¹, *Oliver Mühlemann*²¹Departement für Chemie und Biochemie, University of Bern, Switzerland; Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland; ²Departement für Chemie und Biochemie, University of Bern, Switzerland

Nonsense-mediated mRNA decay (NMD) co-translationally reduces the steady state levels of many physiological mRNAs, hence acting as a post-transcriptional gene regulation mechanism. The fate of the mRNA is decided during translation termination. According to the „unified NMD model“, the interaction of the poly(A)-binding protein (PABPC1) with the eukaryotic release factor 3 (eRF3), bound to the terminating ribosome, typifies normal translation termination. In contrast, if the distance from the termination codon (TC) to the poly(A) tail is too large for the PABPC1-eRF3 interaction to occur efficiently, the recruitment of the SURF complex to the ribosome, through the binding to eRF3 and eRF1, is facilitated instead. This step initiates the cascade of events that result in the rapid decay of the mRNA [1]. This model predicts that spatial rearrangements of the 3'-UTR altering the physical distance between TC and poly(A) tail could serve as a novel post-transcriptional mechanism for gene regulation. However, no physiological mRNAs being regulated by this putative mechanism has been identified so far [2,3]. Therefore, our goal is to identify endogenous mRNAs that are NMD-sensitive under certain conditions but NMD-resistant under other conditions. As an experimental model we have established a human neuroblastoma cell line that can be differentiated in culture into a neuronal-like state and in which we can inactivate NMD by induction of an RNAi-mediated depletion of UPF1. Whole transcriptome analysis was performed by RNA-Seq on undifferentiated and differentiated cells, either with or without UPF1 depletion and the bioinformatics analysis is ongoing. If such putative physiological mRNAs that are differentially regulated by NMD indeed exist, they should be revealed by comparing the transcriptomes under these four conditions. In addition, this analysis will provide new insights into the post-transcriptional regulation in a neuronal-like background.

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442-B Mpn1, mutated in poikiloderma with neutropenia protein 1, unveils cellular surveillance of catalytic spliceosomal small nuclear RNAs.*Vadim Shchepachev*¹, *Charlotte Sonesson*², *Claus M. Azzalin*¹¹ETH Zürich; ²Swiss Institute of Bioinformatics

We have recently demonstrated that Mpn1 is an evolutionary conserved RNA exonuclease that trims the 3'-end polyuridine (oligoU) tail of the spliceosomal small nuclear RNA (snRNA) U6 post-transcriptionally. Fission yeast strains deleted for the *mpn1*⁺ gene and cells from human patients affected by the rare genodermatosis poikiloderma with neutropenia (PN), which is associated to mutation in the *hMPN1* gene, carry aberrant U6 molecules with oligoU tails that are longer than in normal cells and are devoid of their typical 2'-3' cyclic phosphate groups. We have also shown that lack of Mpn1 leads to increased U6 degradation rates, revealing that Mpn1 promotes U6 stability, and proposed the existence of a quality control of U6. Here we show that in *mpn1*⁻ yeast cells, U6 oligoU tails are largely adenylated, indicating that 2'-3' cyclic phosphate groups may prevent adenylation. Adenylated U6 also accrues in cells deleted for the nuclear exosome component *rrp6*⁺ and in double mutants *mpn1*⁻/*rrp6*⁻: thus Rrp6 is at least in part responsible for the degradation of U6 molecules that have not been processed by Mpn1. Ectopically expressed, catalytically inactive U6 molecules are not processed by Mpn1 confirming the existence of a cellular RNA surveillance pathway able to discriminate between catalytically active and inactive U6, and to communicate the outcome of the quality check to Mpn1. We also find that human Mpn1 processes not only U6 but also U6atac, an snRNA component of the minor U12-type spliceosome. Similarly to U6, in PN cells, U6atac lacks a 3' end cyclic phosphate and is degraded more rapidly than in cells from healthy individuals. Our data unveil an intricate cellular circuit dedicated to the quality control of major and minor spliceosome components, and pave the way for understanding the molecular bases of PN.

445-B CBP1 mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner

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Cells have surveillance systems that eliminate aberrant mRNAs to prevent the production of potentially harmful protein products. Non-Stop Decay (NSD) rapidly degrades an aberrant mRNA lacking a termination codon (nonstop mRNA) that is produced mainly by polyadenylation within an ORF. No-Go Decay (NGD) leads to an endonucleolytic cleavage of the mRNA when a ribosome is stalled on the ORF, and produces nonstop mRNA. We recently reported that the Dom34:Hbs1 stimulates degradation of the nonstop mRNA by dissociating the ribosome that is stalled at the 3' end of the mRNA. However, an endonuclease and an endogenous target of NGD have not been identified. tRNA splicing endonuclease is reported to localized to mitochondria outer membrane to play a role of itself, although there is a possibility that it cleaves a cytoplasmic mRNA.

Here, we report that *CBP1* mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner. *CBP1* is a nuclear encoded protein that is imported into mitochondria. Deletion analyses revealed a mitochondria targeting signal and a 652-726nt sequence of *CBP1* are indispensable for the cleavage. Furthermore, a 643-738nt sequence of *CBP1* inserted between a *GFP* ORF and a *HIS3* ORF of a *GFP-HIS3* reporter sufficiently induced the endonucleolytic cleavage when a mitochondria targeting signal was also inserted at the amino terminus of GFP. Also, tRNA splicing endonuclease component *sen54* and *sen2* mutant cells lacking endonuclease activity eliminated the cleavage of *CBP1* and *GFP-HIS3* reporter mRNAs. These results suggest that *CBP1* mRNA is localized to the mitochondria to be cleaved by Sen splicing endonuclease complex. This is the first implication for the correlation between mRNA quality control and mRNA localization to organelle. Now, we are analyzing endogenous mRNAs encoding proteins localized to mitochondria whether to be endonucleolytically cleaved or not by using RNA-seq.

RNA Editing and Modification

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 448 B – 472 B

Location: Main Hallway & Sanada Foyer

- 448 B** **An emerging role for double-stranded RNA binding domains: regulating the sub-cellular localization of proteins**
- 451 B** **MODOMICS: a database of RNA modification pathways - new developments**
- 454 B** **Identification of the last rRNA methylase YhiR E.coli using a new technique**
- 457 B** **Structural and Functional Analysis of Archaeal ATP-dependent RNA Ligase.**
- 460 B** **A Quantitative Atlas of RNA Editing in Mammals Reveals Dynamic Spatiotemporal Regulation**
- 463 B** **The role of ADAR1 in the innate immune response**
- 466 B** **Localization of ADAR1 to cytoplasmic stress granules**
- 469 B** **RNA methylation: a mechanism for post-transcriptional regulation that is deregulated in cancer?**
- 472 B** **RNA-binding proteins regulate substrate-specific changes in A to I editing patterns**

448-B An emerging role for double-stranded RNA binding domains: regulating the sub-cellular localization of proteins

Pierre Barraud¹, Silpi Banerjee³, Stephan Emmerth², Weaam Ibrahim¹, Katrina Woolcock², Marc Bühler², Michael Jantsch³, Frédéric Allain¹

¹ETH Zürich; ²Friedrich Miescher Institute for Biomedical Research Basel; ³Max F. Perutz Laboratories Vienna

Double-stranded RNA binding domains (dsRBDs) are well characterized RNA binding domains that bind specifically to double-stranded RNA (dsRNA) [1]. DsRBD-containing proteins are therefore implicated in many biological processes involving dsRNA such as gene silencing through RNA interference pathways, regulation of translation, RNA processing and messenger-RNA editing. Strikingly, increasing examples of dsRBDs involved in the regulation of protein sub-cellular localization suggest a new important function for a subclass of dsRBDs. For instance, the fission yeast dicer (Dcr1) contains a C-terminal dsRBD that has been shown to be essential for nuclear retention of the protein [2]. Additionally, the third dsRBD of the human mRNA deaminase enzyme ADAR1 has been shown to harbour a nuclear localization signal essential to target ADAR1 to its nuclear substrates [3].

We used solution NMR spectroscopy to get insights into the molecular determinants regulating the sub-cellular localization of these dsRBD-containing proteins. On the one hand, the structure of the C-terminal dsRBD of Dcr1 revealed an extended dsRBD fold embedding an unexpected zinc-binding motif [4]. This unconventional zinc-binding site, which is highly conserved among dicers in yeasts, extends the canonical dsRBD fold thereby generating a conserved and adjustable platform involved in the regulation of the subcellular localization of Dcr1 [5]. Strikingly, although the extended dsRBD of Dcr1 binds to dsRNA, this property is dispensable for proper functioning of Dcr1 in the RNAi pathway. This raises the attractive possibility that this new class of extended dsRBD might generally function in nucleocytoplasmic trafficking and not substrate binding. On the other hand, the structure of ADAR1 dsRBD3 also revealed an extended dsRBD fold with additional structured elements. As in the case of Dcr1, the extension to the canonical dsRBD fold is critical for targeting ADAR1 to the nucleus. Even though it lacks the well-established PY-NLS motif, this extension participates in the interaction with Transportin 1 (also referred to as Karyopherin-β2), thereby promoting ADAR1 nuclear import. As for Dcr1's dsRBD, ADAR1 dsRBD3 fully retains the capacity to bind to dsRNA, suggesting that extending the dsRBD fold would be a widespread strategy to acquire additional function without losing the original dsRNA binding activity.

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[5] Woolcock *et al.* (2012) *Genes Dev.* 26:683-692.

451-B MODOMICS: a database of RNA modification pathways - new developments

Janusz Bujnicki¹, Magdalena Machnicka¹, Kaja Milanowska³, Kristian Rother³, Mark Helm⁴, Henri Grosjean², Elzbieta Purta¹, Okan Osman Oglou⁴, Malgorzata Kurkowska¹, Anna Olchowik¹, Witold Januszewski¹, Stanislaw Dunin-Horkawicz¹, Pawel Piatkowski¹, Sebastian Kalinowski³

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MODOMICS is a database of RNA modifications that provides comprehensive information concerning the chemical structures of modified ribonucleosides, their biosynthetic pathways, location of modified residues in RNA sequences, and RNA-modifying enzymes. In the current database version, accessible at <http://modomics.genesilico.pl>, we included new features: a census of human and yeast snoRNAs involved in RNA-guided RNA modification, a new section covering the 5' end capping process, and a catalogue of "building blocks" for chemical synthesis of a large variety of modified nucleosides. The MODOMICS collections of RNA modifications, RNA-modifying enzymes and modified RNAs have been also updated. A number of newly identified modified ribonucleosides and more than one hundred functionally and structurally characterized proteins from various organisms have been added. In the RNA sequences section, snRNAs and snoRNAs with experimentally mapped modified nucleosides have been added and the current collection of rRNA and tRNA sequences has been substantially enlarged. To facilitate literature searches, each record in MODOMICS has been cross-referenced to other databases and to selected key publications. New options for database searching and querying have been implemented, including a BLAST search of protein sequences and a PARALIGN search of the collected nucleic acid sequences. Finally, we have developed a new nomenclature for nucleic acid modifications.

454-B Identification of the last rRNA methylase YhiR E.coli using a new technique

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The prokaryotic rRNA is highly modified, and one of two major types of these modifications is methylation. The role of methylated nucleotides in ribosome remains undiscovered yet but is still intriguing. The key obstacle arises at the first step of this study: the lack of information about the enzyme that modifies the concrete nucleotide renders the problem practically insoluble.

Here we suggest a very simple and useful method that allowed us to identify the last rRNA methylase YhiR (RlmJ). The proposed method is based on a melting of RNA-DNA duplexes formed by rRNA and two oligodeoxyribonucleotides with different length that are both complementary to rRNA in area of concrete modified nucleotide of rRNA (see figure 1). In our system the shorter oligonucleotide is modified by the Black Hole Quencher (BHQ1) at the 3'-terminus and hybridizes accurately with the nucleotide of interest and some neighboring nucleotides of rRNA. The longer oligonucleotide is modified by FAM at the 5'-terminus and hybridizes with the rRNA just near the shorter one.

We showed that the correct pair of such oligonucleotides allows us to get a remarkable difference in differential melting curves between methylated and non-methylated nucleotide in rRNA.

We proceeded to the search for the last rRNA methylase that modifies nucleotide A2030 of 23S rRNA. The set of annotated and putative rRNA methylases was chosen to be analyzed: YbiN, YecP, YjhP, YafS, SmtA, YmfD, LasT, YfiF, YfiC, YiiV, YecO, YgiQ, KsgA, YhiR, YhdJ and YafE. We compared rRNAs isolated from knockout strains lacking one of listed genes using our melting method and received differential melting curves that showed a great difference in its character for the rRNA from the knockout strain *ΔyhiR* (see figure 2).

Owing to these results we found a very short way to following demonstration of functional activity of the new enzyme. We showed that YhiR is actually SAM-dependent methylase that specifically methylates nucleotide A2030 of 23S rRNA. Moreover, it acts at the first stage of ribosome assembly, when the 23S rRNA is free from ribosomal proteins.

Thus, we demonstrate a very simple and convenient technique for rapid identification of rRNA modifying enzymes.

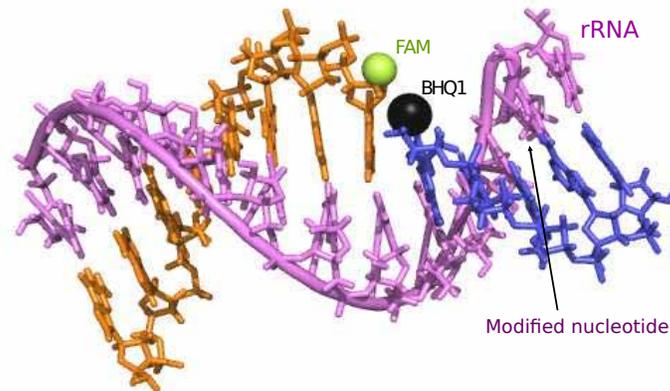


Figure 1. rRNA-DNA duplex. The long oligodeoxyribonucleotide modified by FAM is coloured by orange. The short one is modified by BHQ1 and coloured by blue.

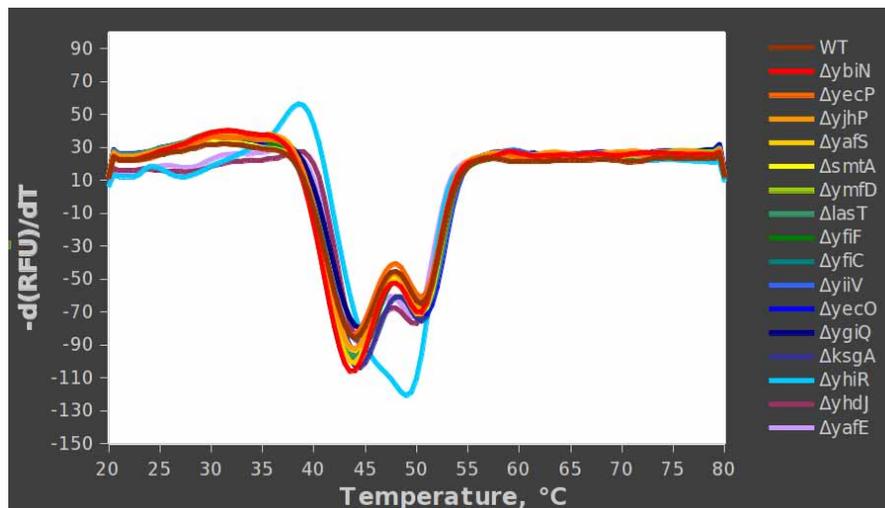


Figure 2. Differential curves for rRNA from corresponding knockout strains and wild type (RFU - relative fluorescence units).

457-B Structural and Functional Analysis of Archaeal ATP-dependent RNA Ligase.*Kiong Ho*¹, *Huiqiong Gu*³, *Yuko Takagi*³, *Katsuhiko Murakami*²¹University at Buffalo, University of Tsukuba; ²Pennsylvania State University; ³University at Buffalo

Archaea possess two different RNA ligases that catalyze the formation of 3'-5' phosphodiester bond; a monomeric RtcB/tRNA ligase that joins 2'-3' cyclic phosphate and 5'-hydroxyl RNA, and a homodimeric ATP-dependent RNA ligase, that joins a 3'-hydroxyl to a 5'-phosphate RNA. Here we report the structure and mechanism of ATP-dependent RNA ligase from *Methanobacterium thermoautotrophicum* (MthRnl). Crystal structure of MthRnl in complex with ATP, AMP-CPP-Mg, and ligase-AMP intermediate were solved. Analysis of mutational effects on individual steps of the ligation pathway underscored how different functional groups come into play during the ligase-adenylation (step 1) reaction versus the subsequent steps of RNA-adenylation (step 2) and phosphodiester formation (step 3), and how does the homodimeric quaternary structure contributes to the ligation reaction. Putative RNA binding surface on the MthRnl was identified through mutagenesis. Mutational analysis within the dimer interface suggests that Phe272, Phe273, Ilu304 and Ilu305 contribute to the stability of the dimer, and contributes to the ligation activity. A proposed model on how MthRnl recognize the break in RNA strands will be discussed.

460-B A Quantitative Atlas of RNA Editing in Mammals Reveals Dynamic Spatiotemporal Regulation*Jin Billy Li¹, Meng How Tan¹, Robert Piskol¹***¹Stanford University Department of Genetics**

RNA editing is as an important post-transcriptional mechanism that diversifies the transcriptome, but accurate, genome-wide measurements of editing levels are lacking, especially in mammals. Here, we build a comprehensive atlas of RNA editing sites in multiple human and mouse normal tissues, different developmental stages, as well as tissues from ADAR1, ADAR2, FMRP, and Pin1 knockout mice. We apply a high throughput microfluidic-based, targeted sequencing approach to capture thousands of human and mouse editing sites identified by us and others. The global patterns discovered in our accurate measurements reveal dynamic regulation of RNA editing. We are carrying out functional assays to interpret the functionality of several editing sites that are differentially edited. Our results are unprecedented at its scale, and will facilitate understanding of how RNA editing is regulated in different biological contexts.

463-B The role of ADAR1 in the innate immune response

*Niamh Mannion*¹, *Sam Greenwood*¹, *Xianghua Li*¹, *Mary O'Connell*¹

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Adenosine deaminases acting on RNA (ADAR) catalyze the conversion of adenosine (A) to inosine (I) within double-stranded RNA (dsRNA). ADARs edit short duplex RNAs site selectively whereas longer dsRNA duplexes are promiscuously edited. A-to-I editing within exons results in recoding events that can alter the functional properties of the encoded proteins. However, the majority of editing events (99% in humans) are promiscuous and occur within non-coding regions of RNA, in *Alu* and other repetitive elements. The biological function of this promiscuous editing is unknown.

In vertebrates there are two catalytically active ADARs; ADAR1 and ADAR2. *Adar1 null* mice die by day E12.5 due to haematopoietic defects resulting from liver disintegration which is associated with an aberrant rise in interferon (IFN). It is not known what unedited substrate(s) causes this lethality in the absence of *Adar1*.

Recently in collaboration with the group of Y. Crow we demonstrate that mutations in ADAR1 cause the human autoimmune disease Aicardi-Goutieres Syndrome (AGS) ¹. Similar to the phenotype in the *Adar null* mice, AGS patients display heightened levels of IFN and increased expression of IFN stimulated genes (ISGs). The catalytic activity of the *ADAR1* mutants were assayed *ex vivo* on a known substrate for ADAR1 and revealed that all of the mutant proteins with the exception of the previously identified catalytically inactive *G1007R* mutation edited the transcript efficiently. The other mutations are in the deaminase domain of ADAR1 but how they cause a disease phenotype is unknown.

We have recently rescued the *Adar1 null* mouse to birth by generating a double homozygous for a gene encoding a key innate immune signalling protein. RNA sequencing and RT-qPCR of immune gene transcript was performed on E11.5 whole embryos and the results reveal that the aberrant rise in type I IFN, inflammatory cytokines and ISGs observed in *Adar1^{-/-}* embryos are rescued in the *double knockout* embryos.

We propose that ADAR1 plays a major role in the regulation of endogenous cellular dsRNAs and in the absence of *ADAR1*, cellular RNAs aberrantly stimulate an innate immune response which leads to autoimmune disease phenotypes such as those seen in AGS patients.

1. Rice, G.I. et al. Mutations in ADAR1 cause Aicardi-Goutieres syndrome associated with a type I interferon signature. *Nat Genet* **44**, 1243-8 (2012).

466-B Localization of ADAR1 to cytoplasmic stress granules*Siew Kit Ng¹, Rebekka Weissbach¹, Deirdre Scadden¹*¹**Department of Biochemistry, University of Cambridge, UK**

Adenosine Deaminases that act on RNA (ADARs) catalyze the conversion of adenosine (A) to inosine (I) within double stranded RNA (dsRNA). Of the three isoforms of ADARs that exist in mammalian cells, only ADAR1 and ADAR2 appear to have catalytic activity. Moreover, differences in subcellular localization suggest that the various ADARs may be involved in different pathways. Analysis of ADAR-null mutants has highlighted the importance of ADARs for normal cell function.

Various studies have demonstrated that ADAR1 p150 plays a role in stress pathways and is essential for survival. Deletion of ADAR1 p150 in mammalian cells results in an embryonic lethal phenotype due to widespread apoptosis. Moreover, a recent study revealed that the loss of ADAR1 p150 also led to global upregulation of interferon-stimulated genes. Apoptosis in the absence of ADAR1 was most likely a consequence of failing to keep the interferon response in check. In addition to these observations, we have recently demonstrated that ADAR1 localizes to cytoplasmic stress granules in mammalian cells following either oxidative stress or interferon induction¹.

We have now used immunofluorescence analyses to investigate which domain(s) of ADAR1 p150 are essential for localization to cytoplasmic stress granules. We have thus demonstrated that the first Z-DNA binding domain (Z α domain), which is uniquely found in ADAR1 p150, is sufficient for localization to stress granules. Furthermore, we have shown that mutation of several key amino acids involved in Z-DNA or Z-RNA binding, as determined from structural analyses, significantly impairs localization. In contrast, the second Z-DNA binding domain (Z β domain), which is unable to independently bind Z-DNA or Z-RNA, is insufficient for localization to stress granules. Importantly, we have shown that editing activity is not required for localization. While unexpected, these observations are in keeping with a previous study that showed that the Z-DNA binding protein 1 (ZBP1) localized to stress granules during various stress conditions. Moreover, we have gone on to show that the Z α domain from ZBP1 is also sufficient for localization to stress granules. We are now further characterizing the requirements for localization of proteins containing Z-DNA (or Z-RNA) binding domains to cytoplasmic stress granules.

¹Weissbach, R. and Scadden, A.D.J. (2012). Tudor-SN and ADAR1 are components of cytoplasmic stress granules. *RNA*, 18, 462–471.

469-B RNA methylation: a mechanism for post-transcriptional regulation that is deregulated in cancer?*Tennille Sibbritt¹, Hardip Patel¹, Brian Parker¹, David Humphreys³, Jeffrey Squires³, Susan Clark², Thomas Preiss¹*¹Genome Biology Department, The John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia; ²Cancer Program, The Garvan Institute of Medical Research, Darlinghurst, NSW, Australia;³Molecular Genetics Department, The Victor Chang Cardiac Research Institute, Darlinghurst, NSW, Australia

Internal methylation of eukaryotic RNA in the form of N6-methyladenosine (m⁶A) and 5-methylcytosine (m⁵C) have been known to exist for decades, however, laborious detection methods have limited the understanding of their role. With the availability of high-throughput sequencing techniques, these drawbacks have been overcome, revealing non-random distribution of internal methylation in a wide variety of RNA biotypes. Early investigation into the prevalence of 5-methylcytosine (m⁵C) in RNA has largely been confined to tRNA and rRNA. Recently, we implemented a bisulfite sequencing-based technique for transcriptome-wide as well as locus-specific detection of m⁵C and mapped thousands of m⁵C sites in the human transcriptome including in mRNA and non-coding RNA. Biased distribution of m⁵C between and within mRNAs, e.g. enrichment in the untranslated regions, is consistent with roles in post-transcriptional regulation of gene expression. m⁵C is a key DNA modification associated with epigenetic gene regulation in mammalian cells and is also known to be deregulated in cancer. We have now begun to investigate the enzymes responsible for modifying mRNA by an RNAi approach as well as a role of RNA methylation in cancer by comparing the m⁵C profiles of normal prostate cells (PrEC) and metastatic prostate cancer cells (LNCaP). Analysis of the recorded patterns of m⁵C sites in mRNA show many transcripts are differentially methylated between each cell line. Currently, we are consolidating and extending the potential link of m⁵C to post-transcriptional regulation and cancer, as well as addressing the molecular function of methylation in mRNA.

472-B RNA-binding proteins regulate substrate-specific changes in A to I editing patterns

Aamira Tariq¹, Wojciech Garncarz², Oliver Pusch⁴, Ales Balik³, Michael Jantsch¹

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RNA-editing by adenosine deaminases that act on RNA (ADARs) converts adenosines to inosines in structured or double-stranded RNAs. As inosines are interpreted as guanosines by most cellular machineries, this type of RNA-editing greatly diversifies the transcriptome, by altering splicing, processing, localization, and translation of coding and non-coding RNAs. Consistently, RNA-editing by ADARs is an essential process in mammals.

Interestingly, editing levels vary in different tissues, during development, and also in pathogenic conditions. Frequently, editing levels do not reflect the levels of ADARs found in a particular tissue or cell type. Hence it appears, as if additional factors may stimulate or repress editing in specific tissues. To isolate such factors we have performed a yeast screen that allows to detect changes in editing via the expression of a reporter gene.

From this screen we have isolated both repressors and enhancers of editing. Three proteins that repress ADAR2 mediated RNA-editing are the RNA-binding proteins RPS14, SFRS9, and DDX15. Overexpression or depletion of these proteins in mammalian cells can decrease or increase editing levels by 15% thus allowing a modulation of RNA editing up to 30%. Interestingly, the three proteins alter RNA-editing in a substrate-specific manner, changing the RNA editing levels of some, but not all editing targets. This substrate specificity correlates well with the RNA-binding preferences of the three proteins.

In mammalian cells, SFRS9 significantly affects editing of the two substrates CFLAR and cyFIP2, while the ribosomal protein RPS14 mostly inhibits editing of cyFIP2 mRNA. The helicase DDX15, in turn, has only a minor effect on mammalian editing substrates. However, lack of DDX15 has a strong effect on the editing of 3' UTRs in *C. elegans*.

Expression of the three factors decreases during mouse brain development nicely showing an inverse correlation with the increase in global editing observed during brain development.

Most interestingly, expression levels of SFRS9 and DDX15 respond strongly to neuronal stimulation or repression of rat brain cultures. The fluctuation of these two factors again correlates nicely with the observed changes in editing levels in repressed or stimulated brain slices.

Colocalization and immunoprecipitation studies demonstrate a direct interaction of SFRS9 and RPS14 with ADAR2, while DDX15 associates with other helicases and splicing factors. Our data show that different editing sites can be specifically altered in their editing pattern by changing the local RNP landscape.

Splicing Mechanisms

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 475 B – 487 B

Location: Main Hallway & Sanada Foyer

475 B **Chemical tools for investigating alternative RNA splicing**

478 B **Role of U2 stem IIb in splicing progression**

481 B **Identification of new natural compounds that modulate splicing in vitro and in cells**

484 B **Conservation of U2 protein – branch site interactions between yeast and human, as investigated by UV crosslinking**

487 B **Functional and structural analysis of Cwc25 required for first-step splicing**

475-B Chemical tools for investigating alternative RNA splicing*Sara De Ornellas*¹, *Ian Eperon*², *Glenn Burley*¹¹University of Strathclyde; ²University of Leicester

Splicing is the processing of pre-mRNA introns and exons to give mRNA for protein biosynthesis. Most eukaryotic genes contain multiple introns and exons. Alternative RNA splicing determines which combinations of exons are spliced, and therefore which protein isoform is synthesised. The variety of the possible isoforms gives rise to the diversity of the proteome; *ca.* 20k protein-coding genes in the human genome give rise to *ca.* 100k transcripts.

Although splicing effects protein diversity and is critical in many genetic diseases, the molecular mechanism of regulation is not well understood. Pre-mRNA contains regions which act as exonic splicing enhancers (ESEs) which bind to SR proteins and enhance splicing. Knowledge of the mechanism of ESEs can help understanding of splice site selection at the molecular level, as well as in the development of therapeutic strategies for the treatment of splicing-related genetic diseases such as spinal muscular atrophy (SMA). Our approach to investigation of these mechanisms focuses on the development and application of novel chemical tools for probing these complex biological systems. Through the development of a new chemical approach, we have recently shown evidence that ESEs do not act via RNA looping mechanisms. Recent results in this area will be presented.

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[2] Lewis, H.; Perrett, A.J.; Burley, G.A.; Eperon, I.C. **An RNA splicing enhancer that does not act by looping.** *Angewandte Chemie International Edition*, **2012**, *39*, 9800-9803.

478-B Role of U2 stem IIb in splicing progression*Alberto Moldon¹, Charles Query¹*¹Albert Einstein College of Medicine

The highly dynamic properties of the spliceosome complicate its structural analysis. Previous studies have reported U2 snRNA conformations representative of different stages in the splicing process. The branch site loop (BSL) is formed at the BS-recognition step, U2 stem IIc and IIa alternate between more stable steps, and U2/6 helices I and II are present during the catalytic steps. Here we study the role of U2 stem IIb. We combined deletions of stem IIb with ATPase/helicase mutants to test for roles at different times and with splicing-defective reporters to detect consequences on the progression of splicing. U2 stem IIb deletion interacted genetically with Prp5 (which is thought to open the BSL), with Prp2 (which activates the catalytic spliceosome) and with Prp22 (which disassembles spliced mRNA from the spliceosome). U2 stem IIb mutants also exacerbated defects of most of the reporters tested.

We also show that there is a complex network of tertiary interactions among stem IIb, nts 26-30 of U2 snRNA, and Prp5, and that stem IIb integrity is needed for Prp5 recruitment. These results indicate a functional relationship among these structures. One hypothesis is that base-pairing interactions between U2 stem IIb and nts 26-30 of U2 compete with other interactions to help disrupt the BSL or U2/6 helix Ia. To better understand this, we designed point mutants to hyperstabilize or destabilize putative alternative conformations formed between stem IIb and nucleotides 26-30 of U2 and analyzed suboptimal splicing reporters. We show that some reporters improve when the alternative structures are stabilized, suggesting a role in opening the catalytic core, whereas others are improved when the alternative structures are destabilized, suggesting roles in promoting stabilization of the complex.

We conclude that U2 stem IIb facilitates opening of the BSL during the BS-recognition step, opening of the catalytic core during transitions between stable conformation, and spliceosome disassembly. This role can be by recruitment of ATPases/helicases that promote the changes, by competition between alternative base-pairing interactions, or both.

481-B Identification of new natural compounds that modulate splicing in vitro and in cells*Andrea Pawellek¹, Ursula Ryder¹, Angus Lamond¹*¹Centre for Gene Regulation & Expression, College of Life Sciences, Dundee, DD1 5EH, Scotland, UK

Pre-mRNA splicing is an important step in gene expression. However in contrast to the other steps involved in gene expression, such as transcription and translation, few specific chemical inhibitors have been characterized that block specific steps in the splicing mechanism and which can be used to dissect the splicing process. Therefore, the identification of specific and selective splicing inhibitors/modifiers would not only be extremely valuable for research purposes as tool compounds, but also potentially useful for therapeutic applications.

To date compounds that have been described to be general splicing inhibitors include: Spliceostatin A (1), Isoginketin (2) and Pladienolide B (3), which are all natural products. In addition, several other natural compounds derived either from extracts of plants, or microbes, have also been reported either to inhibit splicing *in vitro*, or to change splicing of certain transcripts in cells.

We have screened a restricted set of compounds and identified a new group of closely related natural products, typified by the structure we term GRE010, which not only inhibit human pre-mRNA splicing *in vitro* but also alter the splicing of a subset of pre-mRNAs *in vivo* in a variety of different human cells lines. Interestingly, treatment of cells with GRE010 also leads to the specific relocation of early assembly splicing factors within the nucleus, which correlates with their inhibition of splicing and spliceosome assembly *in vitro* at a step after an A complex is formed. We are currently investigating the effect of GRE010 on splicing and cell growth in greater detail.

- 1.) Kaida D et al., Nat Chem Biol. 2007 Sep;3(9):576-83. Epub 2007 Jul 22
- 2.) Kristine O'Brien et al., J Biol Chem. 2008 November 28; 283(48): 33147–33154
- 3.) Kotake Y, et al., Nat Chem Biol. 2007 Sep;3(9):570-5. Epub 2007 Jul 22.

484-B Conservation of U2 protein – branch site interactions between yeast and human, as investigated by UV crosslinking*Cornelius Schneider¹, Klaus Hartmuth¹, Dmitry Agafonov¹, Patrizia Fabrizio¹, Reinhard Lührmann¹***¹Max Planck Institute for Biophysical Chemistry, Göttingen, Germany**

The multiple structural rearrangements occurring during catalytic activation of the spliceosome are only poorly understood at the molecular level. In the early assembly of human spliceosomes the U2 SF3a and SF3b proteins help to recruit the U2 snRNP to the branchsite by engaging in multiple contacts with a region upstream of the branchsite (the so-called "anchoring site"), the branchsite itself and a region downstream of it (Gozani et al., *Genes and Dev.* 2000). While most of the U2 proteins in the yeast *S. cerevisiae* are evolutionarily conserved, it is not clear whether they interact in a similar way with the extended branchsite region in yeast spliceosomes and whether an equivalent to the human anchoring site exists in yeast pre-mRNAs. While it is clear that the affinity of the U2 proteins for the spliceosome is significantly lowered during catalytic activation, it is not known whether this leads to a remodeling of U2 protein-pre-mRNA interactions. To address these questions we have started to investigate protein-RNA interactions by UV crosslinking of purified yeast spliceosomes stalled at specific assembly stages such as the B^{act}, B* or C complex, using our recently developed, purified yeast splicing system (Warkocki et al., *NSMB* 2009). For this purpose we have assembled spliceosomes onto actin-pre-mRNA constructs that harbored either site-specifically ³²P-labeled nucleotides or defined ³²P-labeled RNA stretches, focusing initially on the intronic region in and around the branchsite. Crosslinked spliceosomes were digested with ribonucleases and crosslinked ³²P-labeled proteins were initially identified by 2D gel electrophoresis (Agafonov et al., *MCB* 2011) and by immunoprecipitation. Our results indicate that the region directly upstream of the branchsite is a major interaction platform of the U2 snRNP proteins in yeast B^{act} complexes. Some proteins also crosslink within the branchsite, as well as to the intronic region downstream of it, suggesting that interaction of U2 proteins with the extended branchsite region of the pre-mRNA is conserved in eukaryotes. During catalytic activation and the first step of splicing, we do not observe any significant changes in the interaction pattern. This suggests that the U2 proteins remain in close contact with the extended branchsite region even after the first step of splicing. In addition to U2 SF3a and SF3b proteins, several additional proteins, which are currently being characterized, are crosslinked to the branchsite region in a dynamic manner. We are currently extending our crosslinking approach to study protein interactions with other regions of the pre-mRNA within purified yeast spliceosomes.

487-B Functional and structural analysis of Cwc25 required for first-step splicing*Hui-Fang Wang*¹, *Chi-Kang Tseng*², *Ting-Wei Chiang*², *Soo-Chen Cheng*²¹Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; Faculty of Life Science and Institute of Genomic Science, National Yang-Ming University, Taipei, Taiwan; ²Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Cwc25 is a first-step splicing factor that acts after Prp2 and is recruited to the spliceosome in the presence of Yju2. Previous study shows that Cwc25 with four copies of V5 tagged at the N-terminus has better first-step splicing activity, but binds to the spliceosome more stably when tagged at the C-terminus. These results indicate that tags at different positions of the protein would affect the function of the protein. We therefore systematically analyzed positional and length effects of the tag on Yju2 and Cwc25 on the splicing activity. Splicing was carried out in extracts depleted of both Yju2 and Cwc25, and complemented with different versions of tagged Yju2 and Cwc25. It was found that while splicing was moderately affected when only one protein contained additional residues at the N-terminus, splicing was impaired when both Yju2 and Cwc25 contained additional residues at the N-terminus. We also examined how the structure of Cwc25 affects its function. The N-terminus of Cwc25 is conserved while the C-terminus is highly diverse. A series of C-terminally truncated forms of Cwc25 were constructed. Analysis of the splicing reaction and spliceosome association revealed that deletions of Cwc25 in the C-terminus resulted in destabilization of Cwc25 binding to the spliceosome, suggesting that the C-terminal region of Cwc25 may play a role in stabilizing the association of Cwc25 with the spliceosome.

Splicing Regulation

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 490 B – 529 B

Location: Main Hallway & Sanada Foyer

- 490 B The Evolutionary Landscape of Alternative Splicing and its Regulation in Vertebrate Species
- 493 B The many lives of Co-transcriptional Splicing
- 496 B Damage-induced alternative splicing in MDM2: Identifying cis elements and trans factors
- 499 B Selective constraint on mRNA splicing pattern by protein structural requirement
- 502 B Investigating the role of PTBP1 alternative exon 9 in the evolution of lineage-specific alternative splicing in vertebrates
- 505 B High-resolution Rbfox2 binding patterns predict widespread splicing regulation in mouse embryonic stem cells
- 508 B Effects of SR Protein Expression on HIV-1 Splicing
- 511 B Structural investigation of hnRNP G interaction with SMN RNA
- 514 B Reconstructing alternative splicing of SMN exon 7 by NMR, SRM-Mass-Spectrometry and mathematical modeling
- 517 B Widespread regulatory functions of Polypyrimidine Tract-Binding Proteins in splicing and development of *Arabidopsis thaliana*

- 520 B Mechanisms regulating alternative splicing of Dscam
- 523 B Muscleblind and Fox proteins cooperate to change a splicing program involved in stem cell differentiation and maintenance.

- 526 B EJC can regulate alternative splicing in mammalian cells
- 529 B Regulation of alternative splicing by QKI protein in lung cancer
- 724-B A long non-coding RNA regulates chromatin-mediated modulation of alternative splicing

490-B The Evolutionary Landscape of Alternative Splicing and its Regulation in Vertebrate Species

*Nuno Barbosa-Morais*¹, *Qun Pan*², *Manuel Irimia*², *Serge Gueroussov*⁶, *Eesha Sharma*⁶, *Emil Nachman*⁶, *Hui Xiong*⁷, *Leo Lee*⁷, *Valentina Slobodeniuc*², *Claudia Kutter*⁹, *Stephen Watt*⁹, *Recep Çolak*³, *TaeHyung Kim*³, *Christine Misquitta-Ali*³, *Michael Wilson*⁸, *Philip Kim*⁵, *Duncan Odom*¹⁰, *Brendan Frey*⁴, *Benjamin Blencowe*⁶

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How species with similar repertoires of protein coding genes differ so dramatically at the phenotypic level is poorly understood. Alternative splicing (AS) has been proposed to play an important role in phenotypic differences, because it is a widespread process by which diverse mRNA and protein isoforms can be produced from individual genes. By comparing the transcriptomes of multiple organs from ten vertebrate species spanning ~350 million years of evolution, we observe significant increases in the frequency of "cassette" exon events associated with proximity to the primate lineage [1]. Moreover, in species separated by at least six million years, the exon-skipping profiles of physiologically equivalent organs have diverged to the extent that they are more strongly related to the species identity than to organ type. These species-dependent AS patterns are controlled by a largely conserved cis-regulatory code, together with specific changes in trans-acting factors. In particular, we have identified and characterized species and lineage -classifying "cassette" exon AS events that are predicted to remodel protein-protein interactions involved in gene regulation and other processes. Our recent results indicate that these AS events have further contribute to the major transcriptomic differences underlying phenotypic differences between vertebrate species (refer to abstract by Serge Gueroussov et al.). We have also expanded our evolutionary comparisons to the analysis of other classes of AS events, including alternative retained introns. Intron retention is known to play important roles in the control of mRNA export, localization and turnover by nonsense-mediated decay. Our initial results suggest widespread roles for intron retention throughout vertebrate evolution, and also that this type of AS regulation has impacted the same biological processes and pathways in multiple species. Finally, we are also using our datasets to investigate possible roles of intergenic and antisense non-coding RNAs (ncRNAs) in the regulation of AS. Results from these analyses will be presented.

[1] Barbosa-Morais, N.L. et al (2012). *Science* 338(6114):1587-93

493-B The many lives of Co-transcriptional Splicing*Mattia Brugiolo¹, Lydia Herzel¹, Karla Neugebauer¹*¹**The Max Planck Institute of Molecular Cell Biology and Genetics**

Researchers working in multiple model organisms – notably yeast, insects and mammalian cells – have shown that pre-mRNA can be spliced during the process of transcription (i.e. co-transcriptionally), as well as after transcription termination (i.e. post-transcriptionally). Since 2010, eight studies have used global datasets as counting tools, in order to quantify co-transcriptional intron removal. The consensus view, based on four organisms, is that the majority of splicing events takes place co-transcriptionally in most cells and tissues. We present a summary of the various global datasets and how bioinformatic analyses were conducted. The agreement between budding yeast and higher metazoans indicates that budding yeast is an excellent model for investigating mechanisms of coupling between transcription and splicing. We have previously reported the phenomenon of Terminal Exon Pausing (TEP), in which RNA polymerase II pausing within short last exons correlates with a high degree of co-transcriptional splicing (Carrillo Oesterreich et al 2010 Mol Cell, 40(4):571-581). We have initially focused on potential roles for nucleosome positioning, elongation factors and selected RNA binding proteins. Data on their contribution to TEP and co-transcriptional splicing will be presented.

496-B Damage-induced alternative splicing in MDM2: Identifying cis elements and trans factors*Daniel Comiskey¹, Ravi Singh¹, Aixa Tapia-Santos¹, Dawn Chandler¹*¹The Ohio State University

The *MDM2* oncogene encodes a protein that negatively regulates p53 by targeting it for proteasome-mediated degradation. Through the induction of DNA damage and in cancer, *MDM2* is alternatively spliced into a variety of isoforms. The *MDM2-ALT1* isoform, comprised of exons 3 and 12 is generated in cells in response to genotoxic stress. *MDM2-ALT1* lacks a p53-binding domain and abrogates full-length *MDM2* from binding p53 by sequestering it. This leads to the stabilization of p53, causing cell cycle arrest and/or apoptosis. However, as mutations accumulate to uncouple the p53 pathway, *MDM2-ALT1* becomes an oncogenic driver. For instance, *MDM2-ALT1* is observed in numerous cancers including over 85% of rhabdomyosarcomas and is correlative with high-grade metastatic disease. *It is therefore critical to understand the regulation of stress-induced MDM2 alternative splicing to identify novel targets for anticancer therapies.*

In order to study the alternative splicing of *MDM2* we have developed an *in vitro* splicing system using *MDM2* minigenes and normal and cisplatin-treated HeLa S3 nuclear extracts. The *MDM2* 3-11-12 minigene predominantly excludes exon 11 under UV and cisplatin treatment both *in vivo* and *in vitro*, recapitulating the behavior of the endogenous gene. Using ESEfinder 3.0 we identified predicted binding sites for splicing regulators SC35 and SF2/ASF in exon 11 of the *MDM2* minigene and made mutations in their predicted binding sites to uncover their roles in splicing of *MDM2*.

We confirmed the affinity of these regulatory proteins for their predicted targets through RNA oligonucleotide pull downs using the wild-type and mutant sequences of each binding site. We then performed *in vitro* splicing and demonstrated that disrupting SC35 sites led to exclusion of exon 11 and disrupting the SF2/ASF site promoted inclusion of exon 11. Additionally, overexpression of SF2/ASF in MCF-7 cells led to skipping of exon 11 in our wild-type *MDM2* minigene, while the corresponding site mutant was impervious to SF2/ASF expression. When we knocked down expression of SC35 in SC35 Tet-Off mouse embryonic fibroblasts our wild-type *MDM2* minigene caused exclusion of exon 11 and the corresponding SC35 site mutants did not. We are currently performing splicing assembly assays to determine whether SC35 and SF2/ASF interact with core splicing machinery to facilitate skipping of exon 11 under damage.

To summarize, we have found that SC35 promotes the inclusion of *MDM2* exon 11 under normal conditions, whereas SF2/ASF causes the exclusion of *MDM2* exon 11 under damaged conditions. This is consistent with the canonical role of SC35 as a positive regulator of splicing, but suggests a negative regulatory role for SF2/ASF. Taken together, these data provide insight into the regulation of damage-induced *MDM2* alternative splicing by SC35 and SF2/ASF and present potential targets to modulate *MDM2* alternative splicing in cancer.

499-B Selective constraint on mRNA splicing pattern by protein structural requirement*Jean-Christophe Gelly*¹, *Hsuan-Yu Lin*³, *Alexandre G. de Brevern*¹, *Trees-Juen Chuang*², *Feng-Chi Chen*³¹INSERM, France; ²Academia Sinica, Taiwan; ³National Health Research Institutes, Taiwan

Alternative splicing (AS) of messenger RNA can significantly increase transcriptome diversity in complex organisms. Different AS transcript isoforms can be translated into peptide sequences of different lengths and functions. In mRNA splicing events, whether the splicing patterns are constrained by protein structural requirements remains unclear. Here we address this issue by examining whether the intactness of three-dimensional protein structural units (compact units in protein structures, namely Protein Units (PUs)) tends to be preserved in AS events in human. We show that PUs tend to occur in constitutively spliced exons (CSEs) and to overlap constitutive exon boundaries. In addition, when PUs are located at the boundaries between two alternatively spliced exons (ASEs), these neighboring ASEs tend to co-occur in different transcript isoforms. Moreover, the PU-spanned ASE pairs tend to have a higher frequency of being included in transcript isoforms. ASE regions that overlap with PUs also have lower nonsynonymous-to-synonymous substitution rate ratios than those that do not overlap with PUs, indicating stronger negative selection pressure in PU-overlapped ASE regions. Notably, we found that PUs have protein domain- and structural orderness-independent effects on mRNA splicing. Overall, our results suggest that fine-scale protein structural requirements have significant influences on the splicing patterns of human mRNAs.

Reference: Gelly J-C, Lin HY, de Brevern A.G. *, Chuang TJ*, and **Chen FC***. 2012. Selective Constraint on Human Pre-mRNA Splicing by Protein Structural Properties. *Genome Biology and Evolution* 4(9):966-75.

502-B Investigating the role of PTBP1 alternative exon 9 in the evolution of lineage-specific alternative splicing in vertebrates

*Serge Gueroussov*¹, *Jean-Philippe Lambert*³, *Nuno Barbosa-Morais*², *Anne-Claude Gingras*³, *Benjamin Blencowe*²

¹Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada.; ²Banting and Best Department of Medical Research, Donnelly Centre, University of Toronto, Toronto, Ontario M5S 3E1, Canada.; ³Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto M5G 1X5, Canada.

Through the use of comparative transcriptomics we and others have recently shown that alternative splicing (AS) patterns have diverged dramatically over the course of vertebrate evolution^{1,2} (refer to abstract by Nuno Barbosa-Morais et al.). While the majority of species-specific AS differences appear to be governed by changes in the use of a largely conserved cis-regulatory splicing code, a subset of pronounced species- and lineage-specific AS events are found in trans-acting splicing regulators. These AS events are enriched in disordered regions of proteins and are expected to modulate surface interactions. One such AS event involves mammalian-specific skipping of exon 9 of the splicing regulator PTBP1. This exon encodes a 26 amino acid sequence located within a highly disordered linker region that connects RNA Recognition Motifs (RRMs) 2 and 3. To investigate the functional significance of this lineage-dependent AS event, we generated 293 cell lines that selectively express PTBP1 isoforms with and without exon 9. RNA-Seq profiling of these lines revealed over 100 cassette alternative exons that are differentially-regulated by the two isoforms. Remarkably, the level of splicing of these exons is significantly correlated with the relative expression of the two PTBP1 isoforms across diverse organs and vertebrate species. To investigate the mechanism by which differential inclusion of PTBP1 exon 9 affects lineage-specific AS patterns, we employed isoform-specific immunoprecipitation and quantitative mass spectrometry. Interestingly, exon 9 exclusion results in a several-fold increase in the interaction between PTBP1 and its RRM-containing co-regulator, RAVER1. In summary, a subset of AS differences between vertebrate species can be linked to the differential inclusion of a single alternative exon in the splicing regulator PTBP1. This exon appears to function, at least in part, by modulating the interaction between PTBP1 and its co-regulator RAVER1.

1. Barbosa-Morais, N. et al. 2012. The evolutionary landscape of alternative splicing in vertebrate species. *Science* **338**: 1587–93.

2. Merkin, J., Russell, C., Chen, P., and Burge, C. 2012. Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. *Science* **338**: 1593–9.

505-B High-resolution Rbfox2 binding patterns predict widespread splicing regulation in mouse embryonic stem cells*Mohini Jangi¹, Paul Boutz¹, Phillip Sharp¹*¹**Koch Institute, MIT Department of Biology**

The Rbfox family is unique among splicing regulators, which generally recognize degenerate sequence elements, in its recognition of a well-defined motif, UGCAUG. Yet recent observations imply the existence of additional determinants in defining a functional binding event. Notably, RBFOX2 CLIP(cross-linking immunoprecipitation)-seq in human embryonic stem cells suggested that RBFOX2 occupied only a subset of UGCAUG motifs in expressed transcripts and that not all binding events were motif-dependent (Yeo et al., 2009). Using complementary CLIP-seq and RNA-seq approaches, we explored the relationship between binding and splicing regulation by Rbfox2 in mouse embryonic stem cells (mESCs). To determine Rbfox2-dependent splicing changes in mESCs, we performed RNA-seq upon shRNA-mediated depletion of Rbfox2. Analysis of splicing changes between control and Rbfox2 knockdown using the MISO algorithm (Katz et al., 2010) revealed hundreds of putative Rbfox2-regulated splicing events in all major modes of alternative splicing. Expression of a human RBFOX2 transgene in an Rbfox2 knockdown background resulted in titratable rescue of Rbfox2-dependent splicing changes. We identified direct targets of Rbfox2 regulation in mESCs using a variant of iCLIP (individual-nucleotide resolution CLIP) (Konig et al., 2011; Ule et al., 2005) for epitope-tagged RBFOX2 in mESCs. RBFOX2 binding was enriched in cassette-associated and mutually exclusive exon-associated introns relative to constitutively-spliced introns. Sixty percent of crosslink clusters harbored a motif closely related to the canonical UGCAUG motif. By overlaying RBFOX2 CLIP signal onto Rbfox2-enhanced and –repressed exons determined by MISO, we generated an Rbfox2 RNA map largely consistent with prior observations of position-dependent regulation. We applied the binding criteria inferred from this RNA map to all mouse exons and predicted the regulation of hundreds of additional exons bound by RBFOX2 in flanking introns. Surprisingly, many of these putative Rbfox2-regulated splicing events were predicted to be associated with splicing-coupled nonsense-mediated mRNA decay (NMD). Rbfox2-dependent splicing changes that resulted in unstable mature transcripts may have been underrepresented in our RNA-seq data; however, these events were identifiable in the RBFOX2 CLIP-seq because the crosslink signal originated from pre-mRNA prior to the generation of an NMD isoform. We are currently investigating the extent to which Rbfox2 and the NMD machinery coordinately control isoform expression. We propose that Rbfox2, in part through the regulation of unstable isoforms, orchestrates a more dynamic and widespread network of splicing decisions than previously appreciated.

508-B Effects of SR Protein Expression on HIV-1 Splicing*Le Luo¹, Alan Cochrane², Blanton Tolbert¹***¹Department of Chemistry, Case Western Reserve University; ²Department of Molecular Genetics, University of Toronto**

From a single 9kb polycistronic transcript, HIV-1 generates more than 40 mRNAs, which encode the full viral protein complement. This process is largely regulated by the host protein factors that belong to hnRNP and SR protein families. Generally, hnRNP proteins function to suppress splicing by binding the RNA silencer elements; counteracting hnRNP proteins, SR proteins up-regulate splicing through interaction with RNA enhancer elements. Here, we show that the expression levels of SR proteins modulate the relative abundance of HIV transcript levels. In particular, changes in SRp20 levels (up or down) negatively affect HIV-1 RNA metabolism, resulting in altered balance of RNA splicing that decreases unspliced viral RNA abundance as well as altered splice site selection that modulates Tat expression. Overexpression of 9G8 enhances HIV-1 gene expression while its depletion suppresses synthesis of HIV-1 Gag and, to a lesser extent, Env production. To test whether SRp20 directly or indirectly affects HIV transcript levels, viral RNA IP analysis was performed using a U2OS cell line that stably express non-infectious HIV provirus along with Myc-tagged SRp20 protein. The preliminary result indicates SRp20 specifically binds to HIV transcript. Quantitative experiments are underway to determine the binding preference of SRp20 to the HIV unspliced, singly spliced, and multiply spliced RNA classes.

511-B Structural investigation of hnRNP G interaction with SMN RNA*Ahmed Moursy¹, Antoine Cléry¹, Frederic Allain¹*¹ETH Zürich

The protein hnRNP G is an important regulator of gene expression in human cells. This factor modulates splicing of several pre-mRNAs including Survival of Motor Neuron (SMN). SMN is encoded by two genes SMN1 and SMN2, which differ by five nucleotides. As a result, a different set of splicing factors is recruited on SMN2 resulting in exon 7 skipping in most of its transcripts. Inclusion of this exon is essential for the production of functional SMN proteins⁽¹⁾. A homozygous loss of SMN1 gene results in the insufficient production of active proteins from the SMN2 gene causing the Spinal Muscular Atrophy (SMA) disease. As the SMN2 gene is systematically present in the genome of SMA patients, a promising strategy to cure SMA is to target the splicing of SMN2 transcripts to increase the percentage of exon 7 inclusion and consequently the production of functional SMN proteins⁽²⁾.

hnRNP G was previously proposed to be recruited by Tra2- β 1 upstream its binding site on SMN exon 7⁽³⁾ and together, they activate the inclusion of this exon⁽¹⁾. The specificity of RNA recognition by hnRNP G remains elusive. Based on high sequence identity between the RNA Recognition Motifs (RRMs) of hnRNPG and RBMY, its paralogue in testis that binds CAA containing RNA motifs⁽⁴⁾, it was suggested that hnRNP G could bind similar sequences on SMN exon 7⁽³⁾. In this study, we use NMR spectroscopy to better characterise the mode of RNA recognition of this protein. We solved the structure of hnRNP G RRM in complex with the SMN exon 7 derived 5'-AUCAAA-3' RNA. The structure reveals that the RRM recognises specifically two successive adenines utilizing its β -sheet surface and, more surprisingly, its C-terminal extremity. This mode of recognition differs from what was previously reported for RBMY which, unlike hnRNP G, recognises an additional cytosine 5' to the two adenines⁽⁴⁾. We could show that the recognition of this additional cytosine by RBMY RRM occurs only in the context of interaction with a stem-loop RNA. Interestingly, two stretches of successive adenines are present in the putative hnRNP G binding site on SMN and we tested the importance of hnRNP G binding to them for exon 7 splicing both in vitro and in vivo.

In conclusion, this study reveals the mode of RNA recognition of hnRNP G and brings novel information about regulation of SMN exon7 alternative splicing by this protein.

1. Hofmann Y, et al., Hum. Mol. Genet. 2002. 11(17):2037-49.
2. Lorson CL, et al., Hum. Mol. Genet. 2010. 19(R1):R111-8.
3. Cléry A, et al., Nat. Struct. Mol. Biol. 2011. (4):443-50.
4. Skrisovska L, et al., EMBO Rep. 2007. 8(4):372-9.

514-B Reconstructing alternative splicing of SMN exon 7 by NMR, SRM-Mass-Spectrometry and mathematical modeling

Yaroslav Nikolaev¹, Martin Soste², Paola Picotti², Dagmar Iber³, Frederic Allain¹

¹ETH Zurich, Molecular Biology and Biophysics; ²ETH Zurich, Biochemistry; ³ETH Zurich, Computational Biology

Spinal Muscular Atrophy (SMA) is a lethal neurodegenerative disease affecting human infants with an incidence rate of 1 in 6000 live births. SMA phenotype is strongly connected to skipping of exon 7 in the Survival Motor Neuron 2 (SMN2) gene, which appears to be primarily driven by a single silent mutation in +6 position of the exon (c.840C>T). According to available data this mutation converts binding site of the positive SMN splicing regulator SRSF1 into the site of the negative regulator hnRNP A1 (A1). Despite substantial research efforts the exact molecular mechanisms leading to exon 7 skipping are still poorly understood. Among the reasons for such limited progress is the complexity of exon 7 splicing regulation, which involves interplay of at least 5 regulator proteins.

In this project we aim to explain the mechanism of SMN exon 7 skipping by building a holistic model of this system using Nuclear Magnetic Resonance Spectroscopy (NMR), Selected-Reaction-Monitoring Mass-Spectrometry (SRM-MS) and mathematical modeling. We use SRM-MS to measure the exact *in vivo* concentrations of five regulatory proteins (hnRNPs A1 and G, SRSF1, Tra2b1, SRp30c). Next, we are constructing an *in vitro* model of the system by mixing selectively labelled protein regulators and target SMN RNAs, aiming to visualize all protein-RNA and protein-protein interactions in NMR tube under conditions matching those found *in vivo*. Third, we are integrating the obtained MS, NMR and other biophysical data into a predictive mathematical model to explain the mechanism of exon 7 alternative splicing. Finally, we plan to validate the predictions of the resulting models against exon 7 splicing assays in cell extracts.

Preliminary *in vitro* reconstructions involving three core system components (SRSF1, A1 and target 24nt SMN ESE/ESS RNA) have shown promising results. NMR data indicates that SRSF1 is not completely displaced from SMN2 ESE/ESS sequence under equimolar A1:SRSF1 ratio (100 μ M each). SRM-MS analyses show that nuclear concentration of A1 is 4.5-fold higher than that of SRSF1 (90 μ M A1 vs 20 μ M SRSF1). These facts suggest that increased nuclear concentrations of A1 shall be important for it to drive the exclusion of SMN2 exon 7.

To evaluate possible co-transcriptional effects we performed time-resolved NMR analysis of *in vitro* transcription of the target SMN sequences in the presence of A1 and SRSF1 factors. This analysis shows that folding rate of the target RNA may be slowing down in the presence of regulator proteins, suggesting additional kinetic control in exon 7 alternative splicing.

We expect that extending the core *in vitro* and mathematical models to include remaining regulator proteins will eventually allow predicting the outcome of SMN exon 7 splicing based solely on the knowledge of concentrations of involved regulators.

517-B Widespread regulatory functions of Polypyrimidine Tract-Binding Proteins in splicing and development of *Arabidopsis thaliana*

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The process of alternative splicing (AS) is not only common in mammalian systems, but also a widespread phenomenon in plants, with currently ~60% of all multiexon genes from *Arabidopsis thaliana* being reported to be associated with AS events. Among the regulators of AS are polypyrimidine tract-binding proteins (PTBs), of which three homologues have been identified in *Arabidopsis thaliana*. While the proteins encoded by *At3g01150* (PTB1) and *At5g53180* (PTB2) are closely related, the protein encoded by *At1g43190* (PTB3) exhibits a quite low level of sequence similarity to the other two. Using a misexpression approach for all *Arabidopsis* PTBs to investigate PTB-dependent changes in global splicing patterns, we have identified ~450 putative At-PTB regulation targets, thus providing the first evidence of a widespread AS regulatory role of plant PTBs. Interestingly, a major role in splicing control was only observed for At-PTB1 and At-PTB2, but not At-PTB3. The observed reciprocal changes of AS ratios upon up- and downregulation of At-PTBs indicated a direct regulation of the identified targets by these proteins. Using electrophoretic mobility shift assays (EMSAs), direct RNA/protein interactions could be demonstrated for the target candidate phytochrome interacting factor *PIF6* with At-PTB2. Furthermore, PTB binding motifs within the *PIF6* pre-mRNA have been identified. To address a possible correlation of PTB binding positions and the splicing outcome, the role of polypyrimidine stretches located in different positions around a regulated cassette exon is being examined using a mutational approach.

With respect to the biological functions of plant PTBs, we have reported several flowering time regulators as well as *PIF6*, to exhibit a PTB-dependent alteration of splicing and/or expression patterns. Single T-DNA mutants in *At-PTB1* and *At-PTB2* did not exhibit a visible phenotype, whereas the respective double mutant has been reported to be nonviable. To further elucidate the functional implications of At-PTB1/2, we are aiming to generate plants having the lowest tolerable levels of these splicing factors. Therefore, we are using an approach based on the combination of T-DNA insertion lines and artificial microRNAs (amiRNA). Interestingly, first phenotypical analyses indicated an earlier onset of senescence, as well as stunted growth and a serrated leaf phenotype upon combining the *At-PTB2* knockout with an *At-PTB1* targeting amiRNA.

520-B Mechanisms regulating alternative splicing of Dscam*Matthias Soller¹, Irmgard Hausmann¹, Yash Hemani¹, Pinar Ustaoglu¹*¹**School of Biosciences, The University of Birmingham, Edgbaston, Birmingham, B15 2TT, U.K.**

The *Drosophila* Dscam gene encodes a cell adhesion molecule of the immunoglobulin superfamily and is required for neuronal wiring and phagocytosis in the immune system. A hallmark of the Dscam gene is the extraordinary molecular diversity that can be generated by mutually exclusive alternative splicing in four exon clusters resulting in 38'016 different isoforms. The Dscam splicing pattern has to be different in neighboring mushroom body neurons for normal neuronal development and is altered upon pathogen exposure in immune cells.

To study the mechanisms regulating Dscam mutually exclusive splicing we focused on the exon 9 cluster containing 33 variable exons. We have developed a transgenic fly model containing a reporter gene recapitulating alternative splicing of the endogenous gene. Current models for mutually exclusive splicing in the Dscam exon 6 cluster propose that the variable cluster is kept in a repressed state until one variable exon is chosen. Key to the release of the chosen exon from repression seems to be RNA base pairing between evolutionary conserved sequences in the vicinity of 5' and 3' splice sites of the proximal intron resulting in inclusion of the chosen exon. In the exon 4 and 9 clusters, RNA secondary structure has also been postulated to play an essential role, yet in the distal intron to bring its 5' and 3' splice sites together. Using transgenic flies we are currently testing these models and present our analysis characterizing sequence requirements for exon selection and for mutually exclusive splicing in the exon 9 cluster. Our results will provide important insights into the regulatory mechanisms governing Dscam mutually exclusive splicing.

523-B Muscleblind and Fox proteins cooperate to change a splicing program involved in stem cell differentiation and maintenance.

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Reprogramming somatic cells into induced pluripotent stem cells (iPS cells) types that bear much similarity to Embryonic Stem cells has provided a vast amount of insight into the pathways, mechanisms, and key transcription factors involved in pluripotency. Here we have used human iPS cells derived from normal and senescent fibroblasts to uncover key splicing regulators instrumental in establishing an alternative splicing profile that characterizes pluripotency. Our approach was based on the use of 47 alternative splice events in genes involved in cancer and apoptosis to identify robust alternative changes induced by knock down of selected 49 splicing factors in 5 different cell lines and compare them to similar changes occurring during reversible stem cells induction and re-differentiation. We discovered that two RNA-binding proteins, namely MBNL1 and RBFOX2, when knocked down, accounted for more than 90% of the splicing changes. Strikingly, MBNL1 expression was completely abrogated in stem cells and re-expressed late during mesoderm redifferentiation. We speculate that MBNL1 sequestration by target RNA repeats in cases of Myotonic Dystrophy may impair differentiation of stem cells and contribute to skeletal muscle wasting.

526-B EJC can regulate alternative splicing in mammalian cells*Zhen Wang¹, Valentine Murigneux¹, Hervé Le Hir¹***¹IBENS, 46 rue de l'Ulm, Paris 75005**

The exon junction complex (EJC) is a dynamic multi-protein complex deposited onto nuclear spliced mRNAs 20-24 nucleotides upstream of exon-exon junctions. The four core proteins, eIF4A3, Magoh, Y14 and MLN51 are stably bound to mRNAs during their lifecycle in the cells, serving as a binding platform for other nuclear and cytoplasmic proteins. Therefore, the EJC plays an important role in connecting splicing to downstream post-transcriptional events including mRNA transport, translation and stability. Despite EJCs are deposited onto mRNAs after splicing, recent evidences have shown that the EJC is also involved in splicing regulation of specific events both in *Drosophila* and mammalian cells. However, the mechanism for this new function of EJC in splicing remains largely unknown. To study whether EJC is directly involved in alternative splicing regulation in a genome-wide manner, we performed RNA-seq experiments in HeLa cells with siRNA against EJC components as well as the NMD factor Upf1. Differential expression analysis showed that EJCs affect only a specific set of gene expression changes. Differential exon usage were analysed using MISO and Diffsplice programs, and these identified all types of alternative splicing changes on a global scale in EJC knockdown cells. These splicing changes are specific to EJC core proteins, as knockdown of eIF4A3, Y14 and MLN51 showed the same splicing changes. The splicing changes can be rescued by a siRNA-resistant form of eIF4A3, indicating a direct involvement of EJC core proteins in regulating alternative splicing. Taken together, these data indicate that EJC core proteins are involved directly in alternative splicing regulation on a global scale.

529-B Regulation of alternative splicing by QKI protein in lung cancer*Fengyang Zong¹, Xing Fu¹, Feng Wang¹, Wenjuan Wei¹, Lijuan Cao¹, Hongbin Ji¹, Jingyi Hui¹***¹Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China**

Lung cancer is the leading cause of cancer-related death worldwide. Changes in alternative splicing have been implicated in lung tumorigenesis. However, the functional links between alternative splicing and lung cancer are not well studied. In this study, we observed that RNA-binding protein QKI is down-regulated in non-small cell lung cancer (NSCLC) tissues. Overexpression of QKI in lung cancer cells inhibits cell proliferation and transformation. Using a combined RNAi and RNA-Seq analysis, we identified several hundreds of alternatively spliced genes regulated by QKI and validated at least 24 lung cancer-related events in lung cancer tissues. We have obtained evidence that QKI inhibits cell proliferation through isoform-switch of its targets. To understand the mechanism of splicing regulation by QKI, we generated an RNA map of QKI and revealed that QKI can positively and negatively control exon inclusion in a binding-site position-dependent manner. Additionally, we showed that QKI affects splice site selection by competing with core splicing factors. Our findings demonstrate that QKI regulates a number of splicing events in lung cancer cells and contributes to lung tumorigenesis by modulating alternative splicing of its targets.

724-B A long non-coding RNA regulates chromatin-mediated modulation of alternative splicing*Michelle L¹, Munita R², Misteli T^{3*} and Luco RF^{1*}***¹Institut de Génétique Humaine, CNRS, Montpellier, France, ²Faculty of Biological Science, Pontificia Universidad Católica de Chile, Santiago, Chile; ³National Cancer Institute, NIH, Bethesda, USA**

Recent evidence from numerous laboratories has revealed a novel role for chromatin as a regulator of alternative splicing. Traditionally, chromatin has been suggested to indirectly regulate alternative splicing by modulating the RNA polymerase II elongation rate. We have identified a more direct role of epigenetic modifications in splicing regulation. We find in *FGFR2*, a paradigm of PTB-regulated alternative splicing, distinctive signatures of histone marks that correlate with alternative splicing outcome. Modulation of these modifications induces splice site switching in a predictable manner. The molecular mechanism linking chromatin to the splicing machinery involves a chromatin/splicing adaptor system in which a chromatin-binding protein, MRG15, reads the histone marks and binds specifically to methylated H3K36. In turn, MRG15, by protein-protein interaction, favors the recruitment of the splicing regulator PTB specifically to its RNA splice site, inhibiting exon inclusion. On the other hand, enrichment of H3K27me counteracts the effect of H3K36me in *FGFR2* splicing regulation by inhibiting recruitment of the chromatin-adaptor complex to *FGFR2*. Importantly, we show here that the high H3K27me / low H3K36me chromatin signature is established by a long non-coding RNA antisense to *FGFR2*. This *asFGFR2* promotes recruitment of the Polycomb complex PRC2 and an H3K36 demethylase to *FGFR2*, which creates the splicing-specific chromatin signature characteristic of exon inclusion. These findings identify a lncRNA as a novel class of alternative splicing regulators and they point to cross-talk between histone marks that regulate alternative splicing in a combinatorial way. We propose that the epigenetic memory contained in histone modification patterns is not only used to determine gene activity, but also to establish and maintain cell-specific alternative splicing programs.

RNA-Protein Interactions

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 532 B – 577 B

Location: Main Hallway & Sanada Foyer

- 532 B** Regulation of human telomerase by the helicase RHAU, a quadruplex resolvase.
- 535 B** Dynamic transition upon protein-RNA complex formation: PTB RRM1 interaction with an IRES stem-loop
- 538 B** Control of Mammalian Germ Cell Differentiation by the RNA-Binding Protein DAZL
- 541 B** RNA-binding protein EWSR1 regulates CCDC6
- 544 B** Post-transcriptional regulation of SMN2 expression by hnRNP G and LARP Family proteins
- 547 B** The Runt domain of AML1 (RUNX1) binds a sequence-conserved RNA motif that mimics a DNA element
- 550 B** Computational study of interactions between amino acids and nucleobases in aqueous solvent
- 553 B** Post-transcriptional control of macrophage activation by HuR.
- 556 B** Drosophila Gemin5 binds to UsnRNAs and another specific group of ncRNAs
- 559 B** Neurodegenerative diseases: quantitative predictions of protein-RNA interaction
- 562 B** A co-evolution network of binding contacts between protein L25 and 5S rRNA
- 565 B** Regulation of mRNA metabolism by U2AF65 splicing factor - novel mechanisms for the coordination of gene expression?
- 568 B** Imbalance of Zfp36l2-RNA-binding protein results in female infertility
- 571 B** hnRNP A1 Interacts with Conserved RNA IRES Elements in Enterovirus 71
- 574 B** Tethered Domains and Flexible Regions in tRNase ZL, the Long Form of tRNase Z
- 577 B** Alanine scanning of the aa-tRNA binding interface of Escherishia coli EF-Tu

532-B Regulation of human telomerase by the helicase RHAU, a quadruplex resolvase.*Evan Booy¹, Markus Meier¹, Trushar Patel¹, Ryan Howard¹, Natalie Okun¹, Oksana Marushchak¹, Jörg Stetefeld¹, Sean McKenna¹*¹**Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada**

Telomere extension is mediated by telomerase, an essential eukaryotic reverse transcriptase, and human cells have developmental and tissue-specific strategies for telomerase repression to ensure a defined cellular lifespan¹. Human telomerase is a ribonucleoprotein complex containing a protein component (hTERT) and an essential RNA component (hTR). The 5' region of hTR RNA contains several guanosine rich tracts that form four-stranded tetrad structures stabilized by hydrogen bonds (G-quadruplexes). Previous evidence suggests that a G-quadruplex within this hTR disrupts the formation of an important base-paired structure within hTR known as the P1 helix, a critical element in defining the template boundary for reverse transcription²⁻³. RHAU, also known as DHX36, is an ATP-dependent RNA helicase that belongs to the DExH/D family of RNA modifying enzymes⁴. RHAU possesses the unique ability to preferentially bind and unwind G-quadruplexes⁵. Herein, we present the characterization of the RHAU-hTR quadruplex interaction using biophysical and structural approaches, confirming the importance of the RHAU-specific motif in the interaction with hTR. We demonstrate that the helicase activity of RHAU is sufficient to unwind the quadruplex and promote an interaction with 25 internal nucleotides to form a stable P1 helix, and we have investigated the functional implications of this interaction. Screening of the human transcriptome for novel RNA-quadruplex interaction partners of RHAU identified PITX1 mRNA as a hit, with the protein product being an established transcriptional repressor of hTERT⁶. We present data demonstrating that RNA quadruplexes in the 3'-untranslated region of PITX1 mRNA are RHAU binding sites, and we detail our investigations into the functional mechanisms whereby PITX1 regulation is achieved by RHAU and components of the RNAi machinery. Together, the data implicate the unwinding of RNA quadruplexes by RHAU in multiple facets of telomerase regulation.

References:

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2. Lattmann, S., *et al.* (2011). *Nucleic Acids Res* **39**, 9390-404.
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535-B Dynamic transition upon protein-RNA complex formation: PTB RRM1 interaction with an IRES stem-loop*Fred F. Damberger*¹, *Christophe Maris*¹, *Frédéric H.-T. Allain*¹, *Sapna Ravindranathan*²¹ETH Zurich, Institute of Molecular Biology & Biophysics, Zurich, Switzerland; ²Central NMR Facility, National Chemical Laboratory, Pune 411008, India

We are examining the interaction of the N-terminal RNA recognition motif domain (RRM1) of the Polypyrimidine Tract Binding (PTB) protein with a stem-loop RNA that contains a UCUUU pentaloop present in the Internal Ribosomal Entry Site (IRES) of *Foot-and-Mouth disease virus* (FMDV), *Encyphalomyocarditis virus* (EMCV) and *Theiler's Murine Encephalomyelitis Virus* (TMEV). Where as previous structure determinations of PTB-RRM1 bound to the single-stranded RNA CUCUCU showed that recognition is achieved through canonical RRM-RNA interactions via the β -sheet surface of RRM1 and the loops connecting the β -strands [1], our structure determination of PTB RRM1 bound to the stemloop RNA shows that the C-terminal tail of RRM1 which includes part of the extensive linker connecting RRM1 to RRM2 in PTB forms an additional α -helix which docks to β_2 of the β -sheet. Interestingly the newly formed α -helix makes no contacts to the RNA raising the question of how the C-terminal part of RRM1 is able to sense the binding of a structured RNA to the β -sheet. NMR measurements and site directed mutagenesis at the interface of the C-terminal helix and the β -sheet indicate that RRM1 is in a dynamic equilibrium between conformations where the C-terminal helix is docked to the β -sheet and other conformations where it is not. NMR relaxation dispersion measurements reveal that a dynamic network connects the C-terminal tail with the adjacent β -sheet and remote structural elements which are involved in binding the stemloop. This network which couples binding of RRM1 to a stemloop RNA with formation of an additional secondary structure element shows how PTB can adapt to recognize an ordered RNA target in the context of IRES mediated translation.

[1] Oberstrass F.C. et al. (2005) 309, 2054-2057.

538-B Control of Mammalian Germ Cell Differentiation by the RNA-Binding Protein DAZL*Renaud Desgraz¹, Katherine Romer², David C Page¹***¹Whitehead Institute for Biomedical Research, Howard Hughes Medical Institute and Massachusetts Institute of Technology, Cambridge, MA, 02142; ²Whitehead Institute for Biomedical Research, Howard Hughes Medical Institute and Massachusetts Institute of Technology, Department of Biology, Cambridge, MA, 02142**

Female and male gametes (egg and sperm) differentiate from embryonic germ cells. Translational control by RNA-binding proteins (RBP) is a central mechanism of germ cell biology and many of the germ cell RBPs are essential and conserved across a wide range of species. Despite this conservation, RBP interacting transcripts and the spatiotemporal regulation of these transcripts have remained largely elusive. Mammalian primordial germ cells (PGC) are specified in the proximal epiblast around embryonic day 7.5 and subsequently migrate to the somatic gonad. Upon entering the gonad, PGCs downregulate pluripotency markers and initiate the sex-specific differentiation programs. Female PGCs initiate meiosis before arresting in diplotene whereas male PGCs undergo cell cycle arrest and initiate spermatogenesis after birth. DAZL is a conserved RBP expressed in PGCs when they enter the gonad and its expression in germ cells is essential for both the downregulation of the pluripotency program and the sex-specific cellular differentiation events. *Dazl* gene inactivation in mice leads to embryonic germ cell death and sterility. Despite its central role in germ cell differentiation and survival we ignore the transcripts bound and regulated by DAZL. We have used iCLIP, an unbiased whole genome approach to identify and characterize DAZL-RNA interactions in mammalian germ cells. We show that DAZL-regulated transcripts are enriched for genes encoding cell cycle regulators of all phases of the cell cycle. We also show that DAZL binds preferentially to the 3' UTR of its target transcripts near the stop codon. Finally we have combined single molecule FISH and immunofluorescence to characterize the DAZL-RNA interactions in vivo. DAZL appears to regulate the cell cycle transitions occurring in germ cells by binding to the 3' UTR and promoting the translation of cell cycle regulators but the molecular mechanism regulating DAZL-RNA interactions remains to be addressed and will further our understanding of translational control of germ cell biology and fertility.

541-B RNA-binding protein EWSR1 regulates CCDC6*Sujitha Duggimpudi¹, Erik Larrson², Arndt Borkhardt¹, Jessica Hoell¹***¹Department of Pediatric Oncology, Hematology and Clinical Immunology, Center for Child and Adolescent Health, Heinrich Heine University, Duesseldorf, Germany.; ²Department of Medical Biochemistry and Cell biology, Institute of Biomedicine, The Sahlgrenska Academy, University of Gothenburg, Sweden.**

Dysregulation in the interactions between RNA and RNA-binding proteins (RBPs) by mutations, translocations or over expression results in several diseases. Altered protein expression of the ubiquitously expressed FET family of proteins FUS, EWSR1 and TAF15 has been shown to cause neurological diseases as well as sarcomas and leukemias. PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking & Immunoprecipitation), a technique to study the RNA interactome of any RBP, was applied to the FET family proteins as described in our previous study (Hoell et al, Nat Struct Mol Biol 2011) and global RNA targets of these proteins were defined. In the present study we focussed on EWSR1 which is fused to several DNA binding proteins (e.g. FLI-1, ERG, ETV-1) to form active transcription factors in Ewing Sarcoma.

Ewing Sarcoma is the second most common bone and soft tissue malignancy in adolescents and young adults. Research so far focused nearly exclusively on the above-mentioned fusion proteins and the lost RNA binding capabilities of the C-terminal portion of EWSR1 remained unexplored. In order to study the functional impact of the loss of one wild type allele of EWSR1 on sarcomagenesis, we tested several mRNA targets predicted by PAR-CLIP including MDM2, CCDC6, CFBF and FGF9. Our results showed that there was a clear reduction in the expression levels of all the above selected genes in the EWSR1 knock down samples compared to the controls. Among the selected targets we chose to further investigate CCDC6 (Coiled Coil Domain Containing 6) which showed the strongest regulation.

CCDC6 is important in cell cycle regulation and acts as a check point control for transition of cells from S to G2 phase. It is also predicted to be a tumor suppressor gene and is down regulated in adenocarcinoma of the lung, colorectal carcinoma, thyroid carcinoma and small cell lung cancer. Interplay between CCDC6 and EWSR1 was further explored using forward and reverse genetic approaches in HEK 293 T cells and Ewing Sarcoma cell line MHH-ES-1. Protein levels of CCDC6 were down regulated upon EWSR1 knock down and up regulated upon EWSR1 over expression. Furthermore, EWSR1 knockdown results in decreased proliferation rates and increased cell death compared to the controls hinting at a defective cell cycle progression. Defects in cell cycle and proliferation are often known to trigger carcinomagenesis. Taken together, our results confirm the regulation of CCDC6 by EWSR1 on mRNA and protein level and the role they play in regulating cell cycle which further helps us to understand the underlying mechanisms in the development of Ewing Sarcoma.

544-B Post-transcriptional regulation of SMN2 expression by hnRNP G and LARP Family proteins

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Spinal Muscular Atrophy (SMA) is a recessive autosomal neurodegenerative disease caused by the loss of Survival Motor Neuron 1 gene (*SMN1*). Previous studies have implicated SMN in RNA metabolism, namely in the biosynthesis of small nuclear ribonucleoprotein complexes, and there is evidence that SMN is involved in axonal mRNA transport. Furthermore, in humans there is an almost identical genomic copy of *SMN1*, the *SMN2* gene. *SMN2* contains a silent mutation in exon 7 that greatly impairs splicing, leading to the predominant production of a truncated SMN protein that is thought to be rapidly degraded. Interestingly, SMA severity is inversely correlated to the number of *SMN2* copies present in the genome. Therefore, SMA would be a strong target for the development of new therapies regarding mRNA stability and translation control. It is widely accepted that mRNA stability and translation are greatly influenced by the action of RNA binding proteins and microRNAs that bind untranslated regions of mRNAs. Hence, identification of proteins binding to SMN2 mRNA would provide a greater insight on mechanisms underlying its expression and ultimately uncover novel targets that favor mRNA stabilization and/or increased translation. In this work we have used *in vitro* transcribed biotin-labeled probes covering the 3' UTR of SMN2 mRNA to perform pull-down assays to identify proteins binding its 3'UTR. Several proteins were identified by *peptide-mass fingerprinting*, among which were proteins described as having a role in mRNA metabolism, namely hnRNP G (hnG) and members of the La-Related Protein (LARP) family, including a novel LARP1 isoform. As LARP family members remain poorly studied, we characterized LARP isoform expression in the cell lines used in this work by RT-qPCR, confirming the existence of the novel isoform identified by mass-spec. Overexpression of hnG and LARPs resulted in increased expression of both a luciferase reporter containing the SMN 3'UTR sequence and of the endogenous SMN protein. Characterization of SMN mRNA levels identified different mechanisms of action for these proteins at the level of mRNA stability and translation. In order to further clarify the effect of hnG and LARPs on SMN2 expression, hnG and LARPs were co-transfected with various SMN2-3'UTR deletion mutants fused to *firefly* luciferase. Our data supports a role for hnG and LARPs as positive modulators of SMN mRNA expression.

547-B The Runt domain of AML1 (RUNX1) binds a sequence-conserved RNA motif that mimics a DNA element

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AML1 (RUNX1) is a key transcription factor for hematopoiesis that binds to the Runt-binding double-stranded DNA element (RDE) of target genes through its N-terminal Runt domain. Aberrations in the AML1 gene are frequently found in human leukemia. To better understand AML1 and its potential utility for diagnosis and therapy, we obtained RNA aptamers that bind specifically to the AML1 Runt domain. Enzymatic probing and NMR analyses revealed that Apt1-S, which is a truncated variant of one of the aptamers, has a CACG tetraloop and two stem regions separated by an internal loop. All the isolated aptamers were found to contain the conserved sequence motif 5'-NNCCAC-3' and 5'-GCGMGN'N'-3' (M:A or C; N and N' form Watson-Crick base pairs). The motif contains one AC mismatch and one base bulged out. Mutational analysis of Apt1-S showed that three guanines of the motif are important for Runt binding as are the three guanines of RDE, which are directly recognized by three arginine residues of the Runt domain. Mutational analyses of the Runt domain revealed that the amino acid residues used for Apt1-S binding were similar to those used for RDE binding. Furthermore, the aptamer competed with RDE for binding to the Runt domain *in vitro*. These results demonstrated that the Runt domain of the AML1 protein binds to the motif of the aptamer that mimics DNA. Our findings should provide new insights into RNA function and utility in both basic and applied sciences.

550-B Computational study of interactions between amino acids and nucleobases in aqueous solvent*Matea Hajnic¹, Juan Osorio Iregui¹, Thomas Malzac¹, Bojan Zagrovic¹*¹**Department of Structural and Computational Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria**

The genetic code table provides a universally conserved link between the sequences of cognate mRNA and protein pairs. However, a question, which still remains unanswered, is why a particular nucleotide triplet codes for a particular amino acid? A potential connection between physicochemical properties of codons and cognate amino acids i.e. of mRNA and cognate protein sequences was recently explored^[1]. Surprisingly, it was shown that mRNA coding-sequence pyrimidine content strongly correlates with the average propensity of protein sequences to be solubilized by pyrimidine mimetics (protein polar requirement), hinting at the possibility of complementary binding between these two biopolymers being not only responsible for defining the genetic code, but also playing an important role in present-day cells. To further examine potential interactions between proteins and their mRNAs on a microscopic level, molecular dynamics simulations were employed to derive scales of amino-acid solubility in nucleobase-water solutions. These were then used to obtain proteome-wide correlations of average sequence properties of mRNAs and cognate proteins and to compare them with previously observed ones using the polar requirement scale. Structural and energetic analysis of different amino acids in nucleobase-water solutions revealed that amino acids generally tend to interact more readily with nucleobases than with water molecules, with differing propensities depending on the exact system studied. Taken together, newly generated propensity scales provided proteome-wide correlations between compositional properties of mRNAs and cognate proteins that were comparable in strength to those obtained when the polar requirement scale was used, but differed in direction in several important cases. This allowed us to further explore the mRNA-protein complementarity hypothesis and probe its limits and overall validity.

[1] Hlevnjak M, Polyansky AA, Zagrovic B (2012) Sequence signatures of direct complementarity between mRNAs and cognate proteins on multiple levels. *Nucleic Acids Res.* [Epub ahead of print]

553-B Post-transcriptional control of macrophage activation by HuR.*Panagiota Kafasla*¹, *Orsalia Hazapis*³, *Martin Reczko*³, *Dimitris Kontoyiannis*²¹BSRC “Al. Fleming”, Vari, Greece; ²Biological Sciences Research Center “Al. Fleming, Vari, Greece; ³BSRC “Al. Fleming”, Vari, Greece

Post-transcriptional control of the expression of inflammatory molecules is emerging as an efficient and rapid way to regulate the development and the resolution of inflammation. It requires the assemblies of RNA-binding proteins (RBPs) and non-coding RNAs onto specific elements on their RNA targets in Ribonucleoprotein particles (RNPs) which control mRNA maturation, turnover and translation. One of the key players, with an established role in translation and turnover of inflammatory mediators is the RNA-binding protein HuR. HuR binds to AU-rich elements (AREs), and through this binding it has been suggested to act as a stabiliser of mRNAs, either by positively regulating their translation, or by inhibiting their decay. However, data have emerged suggesting its role as a negative regulator of pathologic inflammation: transgenic systems of inducible and macrophage specific HuR overexpression in mice suppress translation of inflammatory mRNAs (Katsanou et al 2005); mutant mice lacking HuR from the myeloid lineage (*LysCre⁺Elavl1^{fl/fl}*) show exacerbations in the biosynthesis of inflammatory cytokines and chemokines (Yiakouvaki et al 2012). Therefore, HuR appears to be a pleiotropic regulator of the expression of inflammatory mRNAs, a role slightly more complicated than its original assignment as an mRNA stabilizer.

To further understand the role of this RBP in post-transcriptional control of inflammatory gene expression we applied PAR-CLIP on resting and activated bone marrow derived macrophages. Analysis of the RNA targets identified reveals HuR's differential contribution towards the development of macrophage activation.

556-B Drosophila Gemin5 binds to UsnRNAs and another specific group of ncRNAs*Sheila SK Li¹, Jonathan PY Lau¹, Tinyi Chu¹, Brian Qin², Ting-Fung Chan², Terrence CK Lau¹*¹Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR.; ²School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR.

Pre-messenger RNA (pre-mRNA) splicing is an indispensable step in protein synthesis. Human RNA splicing is mediated by spliceosome, the molecular machinery that is comprised of various U small nuclear ribonucleoproteins (UsnRNPs). The UsnRNPs biogenesis is highly regulated by a protein complex called the Survival of Motor Neuron complex (SMN complex). The SMN complex is composed of the SMN protein, sm proteins and Gemin2-8. Gemin5 has been identified as the UsnRNAs-binding protein in SMN complex. *Rigor mortis* (Rig) is the gene homolog of human *Gemin5* in *Drosophila*. It is found highly expressed in the central nervous system of larvae and the ovaries of adult flies. We showed that Rig protein share the same function of human Gemin5 in binding UsnRNAs with high affinity and specificity. By means of RNA immunoprecipitation and next-generation sequencing, we further revealed that Rig binds to another group of non-coding RNAs, suggesting that Rig is involved in other pathways of RNA binding or RNA biogenesis.

559-B Neurodegenerative diseases: quantitative predictions of protein-RNA interaction*Domenica Marchese*^{1,2}, *Davide Cirillo*^{1,2}, *Gian Gaetano Tartaglia*¹¹Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain ²Universitat Pompeu Fabra (UPF), 08003 Barcelona, Spain.

Although neurodegenerative diseases are traditionally described as protein disorders leading to amyloidosis (Rubinsztein 2006; Dobson 1999), very recent evidence indicates that protein-RNA associations are involved in a number of neuropathies. Using our computational method catRAPID (Bellucci et al. 2011) we analyzed interactions between protein and RNA molecules linked to neurodegeneration. In particular, we focused on: 1) *FMRI*, whose codon expansion (55-200 CGG repeats) in the 5' untranslated region of the gene is associated to Fragile X-associated Tremor/Ataxia Syndrome (FXTAS); 2) Iron regulatory protein 1 (IRP1) translational regulation of Alzheimer's and Parkinson's disease related genes through the recognition of IRE (Iron-Responsive Element).

According to our calculations, CGG repeats in the 5'UTR of *FMRI* gene, have a strong propensity to sequester MBNL1 and hnRNP-G and all the proteins (hnRNP-A1, hnRNP-A2/B1, hnRNP-C, hnRNP-D, hnRNP-E and hnRNP-C) that were found to colocalize with CGG repeats by Sellier et al. 2010. In agreement with experimental evidence we also find poor sequestration propensity for FMRP and CUGBP1, while we predict that PURa, which colocalizes with cytoplasmic CGG repeats in flies (Jin et al. 2007) but not in mammalian cells where it is strictly nuclear, interacts with CGG repeats. Interestingly, we predict that SAM68, essential for the recruitment of other proteins but not interacting with CGG repeats from experimental evidence, does not interact with CGG repeats. Nevertheless, among its annotated protein interactors we find that cold-inducible RNA-binding protein CIRBP and polypyrimidine tract-binding protein 2 PTBP2, have a propensity to be sequestered by CGG repeats which increases with its length, thus suggesting that they could mediate SAM68 sequestration.

With regards to Irp1, our method correctly predicts that the 5'-UTRs of ferritin and *APP* interact with IRP-1 in several regions along the protein sequence, which is consistent with experimental evidence (Rogers et al., 2002; Walden et al. 2006). In addition, in line with what reported by Cho et al., we discovered that 87% of the mutations in the CAGAGC motif strongly reduce IRP-1 binding ability. Interestingly, one RNA stem loop within the 5'-UTR of human *a*-synuclein transcript that has been predicted to be structurally related to the IRE element present in ferritin mRNA, has the highest propensity to bind to IRP-1 according to our calculations.

In conclusion, we propose catRAPID for predictions of protein-RNA associations, to flag putative interactions and select candidates for experimental studies. Our method allows processing of a large amount of protein-RNA pairs and can lead to finding previously unknown interactions.

562-B A co-evolution network of binding contacts between protein L25 and 5S rRNA*Zhichao Miao¹, Eric Westhof²*¹Architecture et Réactivité de l'ARN, Institut de biologie moléculaire et cellulaire du CNRS, 67000 Strasbourg France;²Architecture et Réactivité de l'ARN, Université de Strasbourg, Institut de biologie moléculaire et cellulaire du CNRS, 67000 Strasbourg France

RNA-protein interactions are central to all critical processes in the living cells. With the growth in the number of structures of RNA-protein complexes and in sequence data, a systematic analysis and study of RNA-protein interactions could help us understanding recognition mechanisms of RNA-protein binding in order to predict them. Here we chose to study the complex structure of ribosomal protein L25 and 5S rRNA for which detailed structural interactions are available. We analyzed binding and co-evolution patterns between them both on structure and sequence. Mutual Information (MI), a co-evolution analysis, determines residue pairs that show a statistically significant correlations, which result from both direct couplings and multitudinous couplings. Direct-coupling analysis (DCA), can discriminate the two kinds of couplings and give direct information (DI) between residue pairs. We used DCA to characterize co-evolution between L25 and 5S rRNA.

First, we found a highly connected co-evolution network constituted by the RNA binding residues in L25. We applied DCA to L25 and found the result correlates with the structural contact map. Besides, the top co-evolving residue pairs were plotted as a network and clustered. The largest cluster is not only more connected but also includes most of the RNA binding residues. This implies RNA binding may result in co-evolution between RNA binding residues.

Secondly, we found RNA binding residues are separate in the sequence but close in structure. A score is assigned by sequence distance $|i-j|$ divided by the contact distance between residue i and j . It is higher for RNA binding residues than average. RNA-binding was found to cluster on L25 surface and being more conserved than others. A main reason for this observation could be that the local secondary structure features prevent the sequentially close residues from spatial proximity while they are required to cluster in structure to bind to a RNA motif. Thus, L25 needs to separate the RNA binding sites in sequence and gather them in space.

Then, we applied DCA in 5s rRNA, which is the first time it is applied to RNA co-evolution analysis. The results not only show that DI offers less noise than MI but also maps well with RNA structure. The co-evolving nucleotide pairs correlate to most of the base pairs. It validates us DCA could be effective for RNA system and demonstrates its potential in RNA structure prediction.

Finally, DCA was applied between L25 and 5s rRNA. 1424 protein and RNA sequence pairs for different species were collected, aligned and measured by DCA. The top co-evolving residue nucleotide pairs correspond to the key binding sites: five of the top six direct coupling pairs are proximal in structure and three of them map to key hydrogen bonds. The same strategy was extended to L5-5S rRNA and L18-5S rRNA binding, and results were similar. This reveals the great potential of DCA in RNA-protein interaction prediction.

565-B Regulation of mRNA metabolism by U2AF65 splicing factor - novel mechanisms for the coordination of gene expression?*Isabel Peixeiro¹, Samuel Casaca¹, Margarida Gama-Carvalho¹***¹Gene Expression and Bioinformatics Unit, BioFIG – Center for Functional and Integrative Genomics, Faculty of Sciences, University of Lisbon, Portugal**

In eukaryotes, gene expression is a highly controlled process that requires a coordinated regulation at several levels. Post-transcriptional processes can quickly impact cell function through the modulation of mRNA transport, stability and translation. RNA-binding proteins (RBPs) were shown to determine the fate of several mRNA targets, thus affecting multiple cellular processes.

We have previously demonstrated that U2AF65 and PTB, two mammalian splicing factors that recognize pyrimidine tracts, associate with a discrete subset of cellular mRNAs. The functional classification of these interaction profiles revealed underlying mRNA populations encoding proteins involved in common cellular functions. This strongly suggests that their expression is coordinated through overlapping post-transcriptional networks defined by U2AF65 and PTB. In fact, the mRNA population associated with U2AF65 shows a significant enrichment for molecules that encode proteins involved in RNA processing and cell cycle regulation.

Here we characterize the function of U2AF65 as a modulator of the mRNA metabolism by taking advantage of the lambda N-based tethering system. The artificial tethering of U2AF65 downstream of the coding sequence (CDS) resulted in downregulation of the luciferase reporter. Furthermore, we demonstrate that overexpression of U2AF65 has an inhibitory effect on the expression of a reporter gene fusing the luciferase CDS with the 3'UTR of U2AF65, without affecting the reporter mRNA levels. The 3'UTR of U2AF65 presents several predicted U2AF65 binding motifs that bind to recombinant U2AF65. In fact, we show that overexpression of exogenous U2AF65 results in decreased endogenous U2AF65 protein levels, suggesting the existence of a feedback regulatory mechanism. These observations point to U2AF65 as a negative regulator of gene mRNA metabolism, in contrast with its known role as an enhancer of splicing and 3'end processing.

568-B Imbalance of Zfp3612-RNA-binding protein results in female infertility*Silvia Ramos¹*¹University of North Carolina at Chapel Hill

ZFP36L2 is a CCCH tandem zinc finger protein that can destabilize certain AU-rich element-containing transcripts in cell transfection studies. ZFP36L2 has been implicated in the physiological control of female fertility. In the C57/BL6 mouse, a mutation in *Zfp3612* that results in the decreased expression of a form of ZFP36L2 in which the 29 amino terminal amino acids had been deleted, revealed that *DeltaN-Zfp3612* eggs could be fertilized but did not progress beyond the two-cell stage of development. Also when *DeltaN-Zfp3612* females were subjected to superovulation protocols they released 50% fewer eggs than the WT. This suggested a possible additional defect in ovulation and oocyte maturation despite evidence of normal ovarian histology.

To further investigate we introduced the *DeltaN-Zfp3612* mutation into the SV129 mouse strain, which has been reported to respond well to superovulation protocols. *DeltaN-Zfp3612* mutation in this strain also resulted in complete female infertility. Unexpectedly, these females failed to release oocytes using superovulation protocols, prompting us to investigate the oocyte maturation. Remarkably, only 20% of *DeltaN-Zfp3612* oocytes matured *ex vivo*, while 80% of the WT oocytes matured spontaneously. This suggests that *DeltaN-Zfp3612* either inhibits processes involved in the release from, or favor those involved in the maintenance of, meiotic arrest.

The cAMP/PKA pathway is critical for maintaining meiotic arrest. Treatment of *DeltaN-Zfp3612* oocytes with PKA inhibitors doubled the percentage of oocytes able to overcome the *DeltaN-Zfp3612*-linked meiotic arrest. The investigation of the mechanism involved in this arrest, led us to discover that LHR mRNA is the first specific and physiological RNA target for Zfp3612. Decreased expression levels of Zfp3612 in the ovaries resulted in sustained high levels of LHR mRNA during ovulation. Therefore, lack of the normal down regulation of LHR mRNA levels in the ovaries results in anovulation and arrest of the oocytes. This is the first time that a imbalance of a specific mRNA *in vivo* is directly associated with Zfp3612 function. Thus, the Zfp3612-RNA-binding protein can be the basis of some cases of unexplained female infertility in humans.

571-B hnRNP A1 Interacts with Conserved RNA IRES Elements in Enterovirus 71*Michele Tolbert¹, Jeffery Levensgood¹, Mei-Ling Li², Blanton Tolbert¹***¹Case Western Reserve University, Department of Chemistry; ²Robert Wood Johnson Medical School, Department of Molecular Genetics, Microbiology and Immunology**

Enterovirus 71 (EV71) is an emerging threat to public health, there are currently no approved vaccines or treatment. A key step in the early EV71 life cycle is the translation of viral non-structural proteins that initiate viral replication. Due to the lack of m⁷G cap in the viral 5' UTR, EV71 utilizes its own highly structured six stem loop type I IRES to co-opt host proteins. A key IRES trans-acting factor involved in IRES regulation is hnRNP A1. Prior research has shown that hnRNP A1 relocates from the nucleus to the cytoplasm and interacts with Stem Loop II (SLII) of the EV71 IRES, making it likely that SLII is critical in viral IRES dependent activity. Despite its importance in modulating viral IRES mediated translation and thus downstream replication events, little is known about the mechanism of how hnRNP A1 and SLII interact. A series of RNA biophysical studies, bioinformatics, and viral assays were utilized to characterize the two-step binding nature of this interaction. We show that host hnRNP A1 interacts with SLII in a novel manner involving two distinct conserved motifs: (1) a lower five nucleotide bulge containing a canonical UAG hnRNP A1 nucleotide binding motif and (2) an apical six nucleotide hairpin loop containing a CCA hnRNP A1 binding motif. *In vivo* studies further demonstrate that mutation of the UAG motif in the lower bulge abrogates IRES activity and viral replication in host cells. Since it is likely that the structure of SLII modulates this novel binding mechanism, a preliminary high resolution model of SLII was determined. Knowledge of this unique IRES:hnRNP A1 molecular mechanism can pave the way for novel anti-EV71 therapies and treatment.

574-B Tethered Domains and Flexible Regions in tRNase ZL, the Long Form of tRNase Z*Christopher Wilson¹, Emmanuel Chang², Louis Levinger²*¹Brandeis; ²York College of The City University of New York

tRNase Z, a member of the metallo- β -lactamase family, endonucleolytically removes the pre-tRNA 3' trailer in a step central to tRNA maturation. The short form (tRNase Z^S) is the only one found in bacteria and archaeobacteria and is also present in some eukaryotes. The homologous long form (tRNase Z^L), exclusively found in eukaryotes, consists of related amino- and carboxy-domains, suggesting that tRNase Z^L arose from a tandem duplication of tRNase Z^S followed by interdependent divergence of the domains. X-ray crystallographic structures of tRNase Z^S reveal a flexible arm (FA) extruded from the body of tRNase Z remote from the active site that binds tRNA far from the scissile bond. No tRNase Z^L structures have been solved; alternative biophysical studies are therefore needed to illuminate its functional characteristics. Structural analyses of tRNase Z^L performed by limited proteolysis, two dimensional gel electrophoresis and mass spectrometry establish stability of the amino and carboxy domains and flexibility of the FA and inter-domain tether, with implications for tRNase Z^L function.

577-B Alanine scanning of the aa-tRNA binding interface of Escherichia coli EF-Tu

*Emine Yikilmaz*¹, *Olke Uhlenbeck*¹¹Northwestern University

The extensive interface formed between EF-Tu and aa-tRNA includes more than 20 amino acid residues that are highly conserved among bacteria. In order to evaluate the individual contributions of these residues to the thermodynamics of ternary complex formation and their function on the ribosome, 22 point mutations of *E. coli* EF-Tu were prepared that usually changed the native amino acid side chain to alanine. After expression and purification, each mutant protein was activated with GTP, bound to aa-tRNA, and the dissociation rate of the complex (k_{off}) determined by a ribonuclease protection assay (1). While nearly all the mutant proteins were able to form a ternary complex, some of the EF-Tu residues showed aa-tRNA dependent thermodynamic contributions and many of the residues showed decreased binding affinities. The R377A EF-Tu mutant, in which arginine side chain influences secondary structure elements around the tRNA interface, has a faster k_{off} for every aa-tRNA tested. In addition, R223A mutation increases tRNA affinity for aa-tRNAs, presumably by slowing down the conformational changes that lead to dissociation of the ternary complex. Although the effect on binding affinities were quite variable among the different mutations, it is striking that the data closely resembles a similar set of data determined for the ternary complex of *Thermus thermophilus* EF-Tu and Phe-tRNA^{Phe} (2). This indicates that the protein-tRNA interfaces from the two bacteria are not only similar in sequence, but are similar thermodynamically.

We also studied the performance of these mutant proteins in decoding using ribosomes purified from *E. coli* (3). Except for R223A, most of the mutant proteins are active GTPases. It is possible that removal of R223 side chain alters the ribosome binding affinity of the EF-Tu. The ability of each mutant protein to release from aa-tRNA on the ribosome after the GTP hydrolysis step was evaluated using the T1 mutant of Tyr-tRNA^{Tyr}. This tRNA^{Tyr} variant has an altered T-stem that hyperstabilizes binding to native *E. coli* EF-Tu sufficiently that the rate of peptide bond formation (k_{pep}) is extremely slow (4-5). Most of the EF-Tu residues that interact with the 3'-ACCA end and T-stem residues of T1 Tyr-tRNA^{Tyr} are able to stimulate k_{pep} with T1 Tyr-tRNA^{Tyr} compared to wild type EF-Tu. Thus, by weakening binding to aa-tRNA, the EF-Tu mutations are able to overcome the rate limiting block in release of the hyperstabilized aa-tRNA from the protein on the ribosome. This suggests that the energetics of the interface between EF-Tu and aa-tRNA on the ribosome is similar to that of the free ternary complex.

RNP Structure, Function and Biosynthesis

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 580 B – 598 B

Location: Main Hallway & Sanada Foyer

- 580 B** **A genome-wide RNAi screen identifies novel 40S ribosome synthesis factors in human cells**
- 583 B** **Reconstitution of the Uridine-rich snRNP Assembly Machinery**
- 586 B** **Identification of a chloroplast ribonucleoprotein complex containing trans-splicing factors, intron RNAs and novel components**
- 589 B** **Characterisation of the human UTP-B complex and its role in ribosome biogenesis**
- 592 B** **Structural basis of Brr2-Prp8 interaction and its implications for Retinitis Pigmentosa disease type 13 and U5 snRNP biogenesis**
- 595 B** **The rRNA methyltransferase Bud23 interacts with the DEAH-box RNA helicase Ecm16 to promote cleavage at A2**
- 598 B** **THE RIBOSOME-OME I: BIG DATA & THE RIBOSOME**

580-B A genome-wide RNAi screen identifies novel 40S ribosome synthesis factors in human cells

Lukas Badertscher¹, Thomas Wild¹, Lukas Bammert¹, Michael Stebler², Andreas Vonderheit², Christian Montellese¹, Karol Kozak², Gábor Csúcs², Peter Horvath², Ulrike Kutay¹

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Ribosome biogenesis is a complex, highly compartmentalized process that is assisted by more than 150 non-ribosomal proteins, known as trans-acting factors. Our current knowledge on ribosome biogenesis mostly derives from data in yeast, however little is known about this process in mammalian cells.

To shed light on the cellular repertoire of factors involved in ribosome synthesis in mammals, we have performed a genome-wide siRNA screen on 40S subunit biogenesis in HeLa cells using a visual assay relying on RPS2-YFP localization as read-out. Our approach allowed us to compile a list of approximately 400 factors that are required for biogenesis of the 40S subunit. Among the identified proteins are several expected factors, such as the ribosomal proteins themselves, human homologues of yeast ribosome biogenesis factors, as well as splicing factors and nucleoporins. In addition, we identify components of the ubiquitin-proteasome system, of various signaling pathways, metabolic enzymes and uncharacterized proteins.

Of our follow-up studies, we will present data on two different proteins that had not been previously linked to the maturation of ribosomes. One is an uncharacterized protein that we named CRBF1, for which we show that it localizes to nucleoli. Depletion of CRBF1 leads to defects in rRNA processing as demonstrated by FISH and Northern blotting experiments. Proteomic analysis identified CRBF1 as part of a pre-ribosomal particle, indicating a direct function for CRBF1 in ribosome synthesis. The second factor is an enzyme involved in amino acid metabolism that plays a central role in cellular energy homeostasis. Its requirement for ribosome synthesis might potentially explain some of the specific metabolic needs of cancer cell growth.

583-B Reconstitution of the Uridine-rich snRNP Assembly Machinery*Clemens Englbrecht¹, Nils Neuenkirchen¹, Ashwin Chari², Jürgen Ohmer¹, Utz Fischer¹*¹University of Würzburg, Biochemistry, Würzburg, Germany; ²Research Group of 3D Electron Cryomicroscopy, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany

Splicing U snRNPs are the major *trans*-acting factors of the pre-mRNA processing spliceosome. These factors contain a common RNP core composed of seven Sm proteins bound to an snRNA whose assembly is mediated by PRMT5 and SMN complexes. Assembly of snRNPs from RNA and protein is an essential pre-requisite for spliceosome formation. *In vivo*, this is facilitated in a highly complex biogenesis pathway. Initially, the snRNA to be assembled into an snRNP is exported from its site of transcription to the cytoplasm. Here, the assembly of the Sm proteins onto U snRNAs takes place. The snRNP is eventually transported to the nucleus and incorporated into the spliceosome. Several groups including ours have identified a unique machinery that assists the assembly of spliceosomal U snRNPs. This machinery consists of two cooperating units termed SMN-complex and PRMT5-complex, respectively. While the SMN-complex loads Sm proteins onto the snRNA and hence acts as an RNP-assembler, the PRMT5 complex functions upstream in this pathway. It acts as an assembly chaperone by forcing Sm proteins into a higher order structure (termed 6S complex) required for the subsequent transfer onto the SMN-complex. Here we present the total reconstitution of the U snRNP assembly machinery from recombinant sources, which allowed us mechanistic insight into its mode of action. The 6S snRNP assembly intermediate forms in a stepwise manner on the PRMT5 complex and is, upon completion, displaced by pICln or pICln-Sm complexes, thus allowing a new round of 6S formation. The Sm proteins pre-arranged in the 6S complex are then transferred en bloc onto the SMN complex. The SMN complex functions as a scaffold that allows a kinetic proofreading and the association of Sm proteins only with cognate RNA targets. Reconstituted SMN complex containing the SMN(E134K) missense mutation, which is linked to spinal muscular atrophy (SMA), interferes with the RNP-chaperone system and prevents productive U snRNP assembly. Our data reveal an elaborate interplay of U snRNP assembly factors and provide new insight into the cellular defects in SMA.

586-B Identification of a chloroplast ribonucleoprotein complex containing trans-splicing factors, intron RNAs and novel components

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¹**Ruhr-University Bochum, General and Molecular Botany**

Chlamydomonas reinhardtii is widely used for analysis of nucleus-encoded factors that are thought to promote the maturation of chloroplast precursor RNAs. To elucidate the function and composition of ribonucleoprotein complexes that are presumably part of a transcript specific chloroplast spliceosome, we are studying the expression of the chloroplast encoded *psaA* gene [1]. The *psaA* gene is separated into three exons, which are widely distributed over the plastom and flanked by consensus sequences typical for group II introns. The exons are transcribed individually and the major transcript is then assembled in *trans*. Here, we present a novel *trans*-splicing mutant, which is affected in splicing of the first *psaA* intron. Genomic complementation led to the identification of the mutant gene encoding Raa4, a protein of 112.4 kDa, which shares no strong sequence identity with other known proteins [2]. The chloroplast localization of Raa4 was confirmed by confocal fluorescence microscopy, using a GFP-tagged fusion protein. RNA binding-studies showed that Raa4 binds specifically to domains D2 and D3, but not to other conserved domains of the tripartite group II intron. In addition, we used a combined experimental approach including yeast-two hybrid screening, tandem affinity purification (TAP) and mass spectrometry to identify putative interaction partners of Raa4.

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589-B Characterisation of the human UTP-B complex and its role in ribosome biogenesis

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Ribosome biogenesis in eukaryotes requires a multitude of cofactors, many of which are recruited as pre-assembled modules. This has been analysed best for the assembly of early 90S pre-ribosomal intermediates in yeast, where several subcomplexes and RNPs assemble co-transcriptionally on the pre-rRNA. Among them is the UTP-B complex, which in yeast consists of six proteins, Pwp2, Dip2/Utp12, Utp6, Utp13, Utp18, and Utp21.

We are studying the roles of RNA helicases and other cofactors in ribosome biogenesis. Using affinity purification and mass spectrometry to analyse the composition of the human UTP-B complex, we identified homologues of yeast UTP-B proteins as well as candidates for human-specific components of the complex. Among them is the DEAD-box RNA helicase DDX21, for which we have identified putative binding sites on pre-rRNA using UV Crosslinking and Analysis of cDNA (CRAC) and deep sequencing. Interestingly, the potential interaction sites of DDX21 map to rRNA sequences of both the small and the large ribosomal subunit and some of the crosslinking sites cluster in the 3D structure of the ribosome. Preliminary results suggest a role of DDX21 in the regulation of snoRNP binding to pre-ribosomal complexes and it might have other roles in ribosome synthesis.

Taken together, we show that the human UTP-B complex has acquired additional components compared to its yeast counterpart, enabling it to perform more extensive functions in ribosome biogenesis.

592-B Structural basis of Brr2-Prp8 interaction and its implications for Retinitis Pigmentosa disease type 13 and U5 snRNP biogenesis

Thi Hoang Duong Nguyen¹, Jade Li¹, Wojciech P Galej¹, Hiroyuki Oshikane¹, Andrew J Newman¹, Kiyoshi Nagai¹

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U5 small nuclear ribonucleoprotein particle (U5 snRNP) is one of the five canonical subunits of the spliceosome and three of its protein components, Brr2, Prp8 and Snu114, play a crucial role in the formation of the spliceosome's active site. Brr2 is a Ski2-like helicase (246 KDa in yeast), which disrupts the extensively basepaired U4/U6 snRNA duplex and allows U6 snRNA to engage in an intricate RNA interaction network that forms the active centre of the spliceosome. Here we present the structure of yeast Brr2 in complex with the Jab1/MPN domain of Prp8, which has been shown to stimulate Brr2 helicase activity [1]. The residues in the Jab1/MPN domain, whose mutations in human cause the degenerative eye disease Retinitis Pigmentosa type 13 (RP13), are found at or near the interface with Brr2, providing crucial insights into the molecular pathology of RP13. In the cytoplasm Prp8 forms a precursor complex with U5 snRNA, seven Sm proteins, Snu114 and Aar2 but after nuclear import Brr2 replaces Aar2 to form mature U5 snRNP [2]. Together with the recent structure of Prp8⁸⁸⁵⁻²⁴¹³-Aar2 complex [3], our structure provides important insight into U5 snRNP biogenesis.

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595-B The rRNA methyltransferase Bud23 interacts with the DEAH-box RNA helicase Ecm16 to promote cleavage at A2*Richa Sardana¹, Arlen Johnson¹*¹**The University of Texas at Austin**

The small ribosomal subunit assembles co-transcriptionally on the nascent primary transcript. Early processing events at A0, A1 and A2 require U3 snoRNA in the context of the SSU Processome. Cleavage at site A2 releases the pre-40S particle from the primary transcript. We previously identified Bud23 as a conserved eukaryotic methyltransferase responsible for the base modification G1575 in yeast (G1338 in bacteria) and more recently we have found that Bud23 is required for efficient cleavage at A2. Here, we report that Bud23 interacts with the DEAH-box RNA helicase Ecm16 (Dhr1). Ecm16 has previously been implicated in cleavages at A2 and, to a lesser degree, at A1. RNA helicases often require protein cofactors to provide substrate specificity and these interactions are typically through the N- or C-terminal domains of helicases that extend beyond their catalytic cores. We used yeast 2-hybrid analysis to map the binding site of Bud23 to the N-terminal extension of Ecm16. To characterize the particle that Ecm16 acts on, we used a catalytically inactive Ecm16 mutant. Whereas wild-type Ecm16 does not stably associate with preribosomal particles, this mutant does and sediments at approximately 40S. Immunoprecipitation of this mutant reveals a striking accumulation of 21S rRNA and contains U3 snoRNA, Mpp10 and Bud23 among others. 21S rRNA has undergone cleavage at A1 and A3 but not A2. As U3snoRNA is required for cleavages at A0, A1 and A2 these results show that the cleavage pathway has been arrested before A2 cleavage. The accumulation of Mpp10 and Bud23 in the arrested Ecm16 particle is reflected in the altered sedimentation of these proteins, now cosedimenting with Ecm16 at ~40S. These results imply that Bud23 enters the preribosomal particle while U3 is present and shortly before cleavage at A2. Although Bud23 binding is important for 40S biogenesis, its methyltransferase activity is not. We propose that Bud23 recognition of its binding site signals completion of folding of the major domains of the small subunit, coupling Ecm16 activity to assembly status of the subunit.

598-B THE RIBOSOME-OME I: BIG DATA & THE RIBOSOME

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Ribosomes are abundant. Each contains one copy of each of 80 ribosomal proteins (RPs). Therefore, to a first approximation one might expect cells to live with equal numbers of the mRNAs for each of the RPs. The massive transcriptome data for human cells and tissues provides an opportunity to test this assumption. Yet, this expectation has not been satisfied, due in large part to the following problem: each authentic RP gene is diluted by an average of 20 pseudo-RP genes¹, nearly perfect cDNA copies presumably inserted into the genome during the abundant ribosome synthesis of oogenesis. Thus mapping the reads of an RNA-seq analysis against the genome leads to many ambiguous identities. While the various mapping programs deal with this problem in different ways, the result is often an unreliable measure of the actual value, sometimes suggesting 100-fold differences in the level of mRNA for the different RPs.

Modifying the usual mapping systems to provide more accurate assessment of the actual values of RP mRNAs led us to two observations: I) there is substantial variation of the abundance of any given RP mRNA among different cell types, and even among different determinations on the same cell type, e.g., from the ENCODE consortium. II) there is a reproducible difference between the mRNA levels for different RPs, but only over a ~five-fold range. Nevertheless, this result suggests either a substantial regulation of translational rate AND/OR an overexpression of many RPs, with rapid degradation of the excess, as has been recently suggested².

An interesting example is RPL41, that encodes a protein of 24 AA, 17 of which are R or K. It consistently has more mRNA than any other RP. Is this because the efficiency of translation on monosomes is low, or because of the concentration of basic amino acids leads to slow translation.³

Supported by NIGMS RO1 25532

RNA-seq data for multiple tissues was kindly provided by the Gene Expression Applications research group at Illumina, Inc.

¹ Balasubramanian et al *Genome Biology*, **10**:R2, 2009

² Lam, Lamond, Mann, & Anderson *Current Biology* **17**, 749, 2007

³ Charneski & Hurst *PLoS Biology* **11**, e1001508, 2013

RNA Transport and Localization

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 601 B – 607 B

Location: Main Hallway & Sanada Foyer

- 601 B** **Hepatitis B virus post-transcriptional element promotes mRNA export via the cellular mRNA export machinery TREX**
- 604 B** **Growth cone local mRNA translation of nuclear proteins in the spatio-temporal regulation of neurite outgrowth**
- 607 B** **Resolving conflicts between Transcription and Replication: a new potential role for the mRNA export factor Yra1, regulated by its post-translational modifications.**

601-B Hepatitis B virus post-transcriptional element promotes mRNA export via the cellular mRNA export machinery TREX*Binkai Chi¹, Hong Cheng²***¹Institute of Biochemistry and Cell Biology; ²State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China**

In eukaryotes, mRNA export factors are recruited to intron-containing mRNA during splicing, but recruited to intronless transcript probably via specific cis-elements. Hepatitis B viral RNAs are unspliced RNAs that are exported dependent on post-transcriptional element (PRE). However, the mechanism for PRE-mediated mRNA export is still unclear. We found that PRE drastically enhances cytoplasmic accumulation and expression of cDNA transcripts. Systematic deletion analysis indentified two ~100 nt minimum sub-elements in PRE. MS2-MBP affinity purification of proteins bound to these subelements identified TREX components as well as several TREX-associating RNA-binding proteins. Consistent with this result, TREX components efficiently and specifically associate with in vitro transcribed PRE, and this association is both cap- and ATP-dependent. Importantly, knockdown of TREX components and the mRNA export receptor TAP inhibited PRE-mediated mRNA export. Furthermore, by comparison of two functional sub-elements, we identified a 12 nt consensus motif which was sufficient to promote mRNA export when multimerized. Together, our results indicate that PRE enhances the export of intronless mRNA by recruiting TREX. Our study suggests that HBV RNAs competes with cellular mRNAs for cellular mRNA export proteins, facilitates viral RNA export and simultaneously inhibits cellular mRNA export.

604-B Growth cone local mRNA translation of nuclear proteins in the spatio-temporal regulation of neurite outgrowth*Francesca Moretti¹, Olivier Pertz¹***¹Department of Biomedicine, University of Basel**

Neuronal cells exploit local mRNA translation to regulate crucial processes such as neuronal survival, axon pathfinding and synapse formation. Local translation and retrograde transport of transcription factors emerges as a new paradigm to regulate nuclear gene expression in response to signaling events at distal ends of axons and dendrites. We identified a panel of mRNAs that encode nuclear proteins in growth cones of extending neurites (protrusions that are the precursors of axons and dendrites), suggesting that an analogous paradigm might also operate at the very initial phases of neuronal differentiation. We present results on a growth cone localized mRNA encoding a histone-interacting protein with a described role in transcriptional regulation. We show that the knock-down of this mRNA in neuronal cells induces transcriptional changes and impairs neurite outgrowth. Conversely, the overexpression of the encoded protein induces robust neurite outgrowth. Interestingly, the overexpression phenotype is strictly dependent on the presence of the mRNA 3'UTR, which we identified as a determinant that mediates growth cone mRNA localization. We are currently testing whether this protein is locally synthesized in growth cones and retrogradely transported to the cell nucleus. Our initial results suggest that local mRNA translation of a histone-interacting protein might serve as a mechanism to couple the dynamic neurite outgrowth process with transcriptional regulation in the nucleus.

FM acknowledges support from a long-term EMBO postdoctoral fellowship.

607-B Resolving conflicts between Transcription and Replication: a new potential role for the mRNA export factor Yra1, regulated by its post-translational modifications.

Evelina Tutucci¹, Noël Yeh Martin¹, Valentina Infantino¹, Benoît Palancade², Françoise Stutz¹

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The maintenance of genome integrity is one of the most challenging tasks eukaryotic cells have to face. One considerable source of endogenous damage is generated by defects in DNA replication that lead to replication forks stalling and collapse. Particularly during S-phase, cells have to resolve conflicts between replication and transcription, when DNA synthesis is impaired by the RNA polymerase and by factors involved in mRNA processing and export. The mechanism underlying this stress response is not yet elucidated.

Intriguingly, one common factor of these processes is the essential and conserved protein Yra1/REF. Yra1 has a well-characterized role as mRNA export adaptor working in association with the mRNA export receptor Mex67/TAP and the poly(A) binding protein Nab2. We previously reported that Yra1 is a pluri-ubiquitinated protein and we showed that its ubiquitination by the E3 ubiquitin ligase Tom1 is involved in mRNA export regulation (Iglesias, Tutucci et al. 2010). In addition Yra1 is found at origins of replication, but its function in this context is unknown (Swaminathan, Kile et al. 2007).

Our recent work suggests a new role for the mRNA export factor Yra1 in the regulation of cell cycle progression, and shows that Yra1 ubiquitination and in turn Yra1 levels, are sensitive to replication stress caused by different DNA damaging factors. Moreover, we observed that Yra1 is a sumoylated protein and we identified the SUMO-dependent Slx5/Slx8 complex as the second E3 ubiquitin ligase complex involved in Yra1 post-translational modification. Interestingly this ubiquitin-ligase complex binds the DNA and deletion of either gene is paralleled by a general increase in genomic instability (Nagai, Dubrana et al. 2008).

With this work we propose Yra1 as a new factor involved in genome integrity maintenance and we suggest that Yra1 is a sensor of DNA damage and a player in the S-phase stress response. In addition, the fact that Yra1 is present both at the transcription sites and at origins of replication, suggests a role for this protein in resolving conflicts between transcription and replication during S-phase.

Iglesias, N., E. Tutucci, et al. (2010). "Ubiquitin-mediated mRNP dynamics and surveillance prior to budding yeast mRNA export." *Genes Dev* 24(17):1927-1938.

Nagai, S., K. Dubrana, et al. (2008). "Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase." *Science* 322(5901): 597-602.

Swaminathan, S., A. C. Kile, et al. (2007). "Yra1 is required for S phase entry and affects Dia2 binding to replication origins." *Mol Cell Biol* 27(13): 4674-4684.

RNAs in Diseases

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 610 B – 643 B

Location: Main Hallway & Sanada Foyer

- 610 B** S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1
- 613 B** Retinitis pigmentosa mutations of hBrr2 reduce splicing fidelity
- 616 B** Overexpression of miR-29b and miR-122 in the invasive ductal carcinoma of the breast
- 619 B** MECHANISMS OF POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION IN DEMENTIAS
- 622 B** The role of the simtron, miR-1225, and its host gene, PKD1, in autosomal dominant polycystic kidney disease
- 625 B** The FUS protein is required for cell proliferation
- 628 B** Uncovering the role of microRNAs in SMA
- 631 B** A novel function for MiR-10a in the pathogenesis of atopic dermatitis
- 634 B** Knockdown of human Dyskerin, the protein linked to Dyskeratosis congenita, blocks large ribosomal subunit production and activates p53 via RPL5 and RPL11
- 637 B** TDRD3 recruits FMRP and the topoisomerase TOP3 β to spliced mRNAs and provides a molecular link between schizophrenia and fragile X syndrome
- 640 B** Investigating the role and regulation of microRNA-10a in Acute Myeloid Leukaemia bearing the Nucleophosmin1 mutation
- 643 B** Endogenous shRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells with High Purity.

610-B S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1*Vered Ben-Hur¹, Polina Denichenko¹, Zahava Siegfried¹, Avi Maimon¹, Adrian Krainer², Ben Davidson³, Rotem Karni¹***¹Department of Biochemistry and Molecular Biology, Hebrew University-Hadassah Medical School; ²Cold Spring Harbor Laboratory, NY; ³Oslo University Hospital, Norwegian Radium Hospital**

Ribosomal S6 Kinase 1 (S6K1) is a major mTOR downstream signaling molecule which regulates cell size and translation efficiency. Here we report that short isoforms of S6K1 are over-produced in breast cancer cell lines and tumors. Overexpression of S6K1 short isoforms induces transformation of human breast epithelial cells. The long S6K1 variant (Iso-1) induced opposite effects: It inhibits Ras-induced transformation and tumor formation, while its knockdown or knockout induced transformation, suggesting that Iso-1 has a tumor suppressor activity. We further found that S6K1 short isoforms bind and activate mTORC1, elevating 4E-BP1 phosphorylation, cap-dependent translation and Mcl-1 protein levels. Both a phosphorylation-defective 4E-BP1 mutant and the mTORC1 inhibitor rapamycin partially blocked the oncogenic effects of S6K1 short isoforms, suggesting that these are mediated by mTORC1 and 4E-BP1. Thus, alternative splicing of S6K1 acts as a molecular switch in breast cancer cells elevating oncogenic isoforms that activate mTORC1.

613-B Retinitis pigmentosa mutations of hBrr2 reduce splicing fidelity*Zuzana Cvacková¹, Daniel Mateju¹, David Stanek¹*¹Laboratory of RNA Biology, Institute of Molecular Genetics ASCR

hBrr2 is a DExD/H-box RNA helicase crucial for pre-mRNA splicing and its mutations cause autosomal dominant retinal disorder retinitis pigmentosa. In this study, we prepared S1087L and R1090L mutations of human Brr2 using BAC recombineering and expressed them stably in human cell culture. Mutations in hBrr2 did not compromise snRNP assembly and both mutants were incorporated into the spliceosome as the wild-type protein. Surprisingly, cells expressing RP mutants exhibited increased splicing efficiency of the LDHA gene. Next, we found that depletion of endogenous hBrr2 enhanced usage of a cryptic splice site while splicing at the correct splice site was inhibited. Proper splicing of optimal and cryptic splice sites was restored in cells expressing wild-type hBrr2 but not in cells expressing the RP mutants. Taken together, our data suggest that hBrr2 is an important factor in splice site recognition and that the RP-linked mutations S1087L and R1090L affect this hBrr2 function, possibly leading to the increased usage of cryptic splice-sites.

616-B Overexpression of miR-29b and miR-122 in the invasive ductal carcinoma of the breast*Patricia R Darin¹, Juliana S Zanetti², Alfredo Ribeiro-Silva², Fernando L De Lucca¹*¹Department of Biochemistry and Immunology, School of Medicine, University of São Paulo, Ribeirão Preto, SP., Brazil;²Department of Pathology and Legal Medicine, School of Medicine, University of São Paulo, Ribeirão Preto, SP., Brazil

MicroRNAs (miRNAs) are key regulators of gene expression in eukaryotic cells. Current computational predictions suggest that miRNAs regulate the expression of 30% of human protein-coding genes. Several studies indicate that miRNAs regulate genes of signaling pathways involved in important cellular processes, including cell proliferation and apoptosis. The pro-apoptotic RNA-dependent protein kinase (PKR) signaling pathway is activated by PACT (protein activator of PKR). Recently, PACT was shown to be a target of miR-29b and miR-122 and the role played by these miRNAs in the breast cancer is still unknown. The aim of this study was to investigate whether the expression of miR-29b and miR-122 is deregulated in the invasive ductal carcinoma (IDC) of the breast using Formalin-Fixed, Paraffin-Embedded (FFPE) tissues. The expression of miRNAs and PACT was evaluated by real time PCR and immunohistochemistry, respectively. The normal breast FFPE samples were used as control. Our results showed an upregulation of miR-29b (8-fold; $p < 0.01$) and miR-122 (19-fold; $p < 0.005$) in the FFPE samples of IDC. We also observed that PACT expression was reduced by ~ 50% which is consistent with previous studies showing that miR-29b and miR-122 negatively regulate this PKR activator. Thus, the reduction of PACT expression may be accompanied by the decrease of the pro-apoptotic PKR pathway activation. Taken together, our results suggest that the overexpression of miR-29b and miR-122 in IDC is contributing for the inhibition of apoptosis and, therefore, these miRNAs may be involved in progression of the human breast cancer.

The research has been supported by FAPESP.

619-B MECHANISMS OF POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION IN DEMENTIAS*Francesca Fontana¹, Margherita Grasso¹, Valerio Del Vescovo¹, Giuseppina Covello¹, Michela Alessandra Denti¹*¹**Cibio-Centre for Integrative Biology**

MicroRNAs (miRNAs) are noncoding RNA of 18-25 nt, capable of regulating mRNA translation and gene expression at the post-transcriptional level. MiRNAs mis-expression is often associated with human diseases, such as cancers and neurodegenerative pathology conditions. The main objective of this study is an analysis of the post-transcriptional regulation by miRNAs of two important genes, MAPT and PGRN, involved in Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). This is one of the major degenerative dementia syndromes, characterized by atrophy of the prefrontal and anterior temporal lobes. Several studies identified 43 pathogenic mutations in MAPT, that encodes for microtubule associated tau protein. In the brain tau protein has important functions in microtubule (MT) assembly, stability and has a relevant role in neurogenesis, axonal maintenance and transport. The disruption of its function has devastating effects on neuronal integrity and in most neurodegenerative diseases forms neurofibrillary tangles (NFT) accumulation, that trigger neuronal loss. Recently, the discovery of 56 mutations in the progranulin gene (PGRN) explained the heterogeneity of patients affected by FTDP-17. PGRN encodes a secreted precursor protein called progranulin, that is expressed in neurons, microglia and represents an important growth factor involved in the regulation of multiple processes. MiRNAs may be a contributing factor in neurodegenerative diseases. This project focuses on the expression of selected miRNAs in cell lines and primary neurons and on the validation of the miRNAs targeting of PGRN and MAPT 3'UTR. Moreover, we aimed at understanding the influence of SNPs in PGRN 3'UTR on the effect of validated miRNAs and finding if there is a causal role of miRNA deregulation in FTD, through cell systems and mice brains.

622-B The role of the simtron, miR-1225, and its host gene, PKD1, in autosomal dominant polycystic kidney disease

*Mallory A. Havens*¹, *Frank Rigo*², *Michelle L. Hastings*¹

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the leading genetic cause of end stage renal disease and affects 1:1000 individuals worldwide. Currently, there is no cure for ADPKD. ADPKD has variable severity and heterogeneity, which emphasizes the need for the identification of disease modifiers and new therapeutic targets. The gene *PKD1* is responsible of 85% of ADPKD cases and contains the microRNA miR-1225 within highly conserved intron 45. However, the role of miR-1225 has not been examined in ADPKD pathogenesis. Here, we investigate the coexpression of miR-1225 and its host gene, *PKD1* and the potential functional role of miR-1225 in ADPKD. We find that miR-1225 expression is regulated in a tissue-specific manner in humans and is inversely related to *PKD1* expression during mouse kidney development. Also, we discovered that the SR protein SRSF5 is a regulator of miR-1225 biogenesis. In order to investigate the role of miR-1225 in ADPKD, we tested human pathogenic mutations in *PKD1* intron 45 and determined their affect on miR-1225 abundance and mRNA splicing. To further elucidate a possible role for miR-1225 in ADPKD *in vivo*, we designed antisense oligonucleotides (ASOs) that alter miR-1225 abundance without affecting *PKD1* pre-mRNA splicing and are testing them in mice. These data suggest a potential role for miR-1225 in disease pathogenesis along with its host gene, *PKD1*.

625-B The FUS protein is required for cell proliferation

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FUS/TLS (fused in sarcoma/translocated in liposarcoma) protein, a ubiquitously expressed and highly conserved RNA binding protein, has been linked to a variety of cellular processes from mRNA processing to DNA repair. However, the precise function of FUS is not well understood. Recently, mutations in the FUS gene have been identified in familial and sporadic patients of Amyotrophic Lateral Sclerosis, a fatal neurodegenerative disorder characterized by dysfunction and death of motor neurons.

Based on the observation that some mutations in the FUS gene induce cytoplasmic accumulation of FUS aggregates, we decided to explore a loss-of-function situation (i.e. inhibition of FUS' nuclear function) to unravel the role of this protein. To this purpose, we have generated a SH-SY5Y human neuroblastoma cell line which expresses a doxycycline induced shRNA targeting FUS that efficiently depletes the protein. In order to characterize this cell line, we have characterized the poly(A) fraction by RNA deep sequencing. Preliminary results show that FUS depletion affects both mRNA expression and alternative splicing. Upon FUS depletion 330 genes are downregulated and 81 are upregulated. We also found that 395 splicing isoforms were downregulated, while 426 were upregulated. Currently, we are focusing our attention on the pathways which are mostly affected by FUS depletion. In addition, we are currently characterizing how FUS depletion affects cell proliferation and survival. We find that the lack of FUS impairs cell proliferation but does not induce apoptosis.

Finally, since MEFs and B-lymphocytes derived from FUS knockdown mice display major sensitivity to ionizing radiation and chromosomal aberrations [1,2], we are exploring the effects of DNA damage in FUS-depleted cells by monitoring important components of DNA Damage Response (DDR). Taken together, these studies may contribute to our knowledge of the role of FUS in these cellular processes and will allow us to draw a clearer picture of mechanisms of neurodegenerative diseases.

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[2] Hicks GG. et al. (2000) Nat Genet. 2000 Feb;24(2):175-9.

628-B Uncovering the role of microRNAs in SMA*Philipp Odermatt¹, Daniel Schümperli²*¹University of Bern; ²Institute of Cell Biology, University of Bern

Spinal muscular atrophy (SMA) is caused by low level of the survival of motor neuron protein (SMN). As a consequence, the a-motor neurons of the spinal cord die causing muscle weakness and paralysis. SMN is ubiquitously expressed and involved in the assembly and maturation of small nuclear ribonucleoproteins (snRNPs) and is thus essential for survival of all cells. The question why low levels of SMN cause a motor neuron-specific disease such as SMA still needs to be answered. An attractive hypothesis is that SMN may help to regulate the transport, translation or stability of mRNAs that are localised to the axon and that a defect in this function may be the primary cause of SMA. In neurons, translationally repressed mRNAs are transported along the dendrites as part of ribonucleoproteins particles (mRNPs). These mRNAs are kept in a translationally repressed state by several factors, including miRNAs. Upon synaptic activation, the miRNAs dissociate from their targets, and local protein synthesis takes place. My aim is to see whether SMN could interplay with miRNAs and regulate the activity of moto-axonal mRNPs. To investigate these aspects, an inducible human neuroblastoma SMN knock down cell line was created and micro RNA levels of certain miRNA candidates (based on a literature search) are being monitored by qPCR under control and knock down conditions. Preliminary results showed only little changes of the candidate micro RNAs in the cell line model. In a next step, motor neurons will be extracted by laser micro dissection from the severe mouse model and deep sequencing analysis will be performed to analyse the miRNA repertoire in these cells. This should help to define the repertoire of miRNAs affected by the loss of SMN in the severe SMA mouse. The localization of these candidate miRNAs in the cells will then additionally be monitored by miRNA in situ hybridisation in the cell line model and in slice cultures.

631-B A novel function for MiR-10a in the pathogenesis of atopic dermatitis*Toomas Runnel¹, Maya Zimmermann³, Külli Kingo², Cezmi A. Akdis³, Ana Rebane⁴*

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MiR-10 family genes, including miR-10a, are located in the Hox gene clusters and are known to be the regulators for Hox genes, suggesting they might have a role in fetal development. miR-10a is almost nonexistent in embryonic stem cell lines hES1 and hES2 and embryonic carcinoma Ep2102 line. miR-10a expression starts to increase during endodermal differentiation and is quite high in both embryonic and normal adult liver as well as several other inner organs. Additionally, miR-10a has been reported to be expressed in the skin, in conventional and induced regulatory T cells, where it is induced by retinoic acid and TGF- β . Besides other functions, retinoic acid and TGF- β control cell proliferation and promote regulatory T cell differentiation.

Our results demonstrate that miR-10a is upregulated in keratinocytes and skin biopsies from atopic dermatitis (AD) patients compared to healthy individuals. This finding led us to investigate further, whether miR-10a would be an important player in the pathogenesis of AD. First, we stimulated primary keratinocytes and peripheral blood mononuclear cells (PBMCs) with various pro-inflammatory cytokines, TGF- β , retinoic acid, histamine and their combinations. Histamine occurs naturally in mast cells and basophils, and is released upon allergen cross-linking of IgE antibodies and then triggers an inflammatory response. Our experiments revealed miR-10a to be upregulated by histamine in primary keratinocytes, but not by retinoic acid, TGF- β or pro-inflammatory cytokines. In PBMCs, retinoic acid stimulation induced notable upregulation of miR-10a, which was further enhanced by costimulation with TGF- β , whereas histamine treatment had no significant effect. To study the function of miR-10a in inflammatory response of KCs, we overexpressed miR-10a in KCs and observed downregulation of IRAK1 and IL-8 mRNAs. IRAK1 and IL-8 are both mediators of innate immune response involved in regulation of NF- κ B pathway, but are not predicted as direct targets of miR-10a. Computational target prediction with Targetscan revealed transcription factor cAMP responsive element binding protein 1, also known as CREB1, and several MAP Kinase pathway genes as relevant and evolutionally conserved targets of miR-10a. Currently, we are studying the function of miR-10a in keratinocytes and verifying the effect of miR-10a on putative targets. In conclusion, our data suggest that miR-10a has anti-inflammatory function in keratinocytes and might influence the development of AD.

634-B Knockdown of human Dyskerin, the protein linked to Dyskeratosis congenita, blocks large ribosomal subunit production and activates p53 via RPL5 and RPL11*Katherine Sloan¹, Andria Pelava¹, Claudia Schneider¹, Nicholas Watkins¹*¹Newcastle University

Dyskerin (Cbf5), NOP10, NHP2 and GAR1 are core components of H/ACA snoRNPs and as such, are important for ribosome biogenesis. In yeast, most H/ACA snoRNPs catalyse rRNA pseudouridylation, but two complexes, snR30 and snR10, are instead required for 18S rRNA processing. Utp23 is an snR30-specific protein that is also essential for just small subunit biogenesis. In humans, H/ACA snoRNPs are important for pseudouridylation although little is known about their role in pre-rRNA processing. The human H/ACA snoRNP proteins are also associated with scaRNAs (snRNA modification) and the telomerase complex (telomere maintenance). Mutations in Dyskerin, NOP10 and NHP2 result in a genetic disease, Dyskeratosis congenita (DKC). DKC patients have shorter telomeres, consistent with a telomerase defect. There is, however, evidence that this disease may also arise due to a defect in ribosome biogenesis.

The interaction between the essential yeast 18S rRNA processing factor, Utp23 and snR30 (U17 snoRNA in humans) is conserved in human cells. We now show that human UTP23 is also required for 18S, but not 28S and 5.8S, rRNA processing. In contrast, knockdown of the human H/ACA snoRNP proteins surprisingly lead to a block in the production of the large ribosomal subunit (LSU) RNAs, 5.8S and 28S, but not 18S rRNA processing. Northern blot analysis revealed that depletion of Dyskerin, NOP10, NHP2 or GAR1 blocks the primary cleavage in ITS1 at site 2. Similar defects are seen after the knockdown of LSU biogenesis factors, such as BOP1 or RBM28. Our data therefore suggest that the function of human UTP23 is separate from that of the H/ACA snoRNPs.

Several genetic diseases, such as Diamond Blackfan anemia, are due to defects in ribosome biogenesis. These defects are proposed to result in the accumulation of free ribosomal proteins, RPL5 and RPL11, which bind and suppress the activity of HDM2, a suppressor of p53. We show that knockdown of Dyskerin in MCF7 cells leads p53 activation in an RPL5- and RPL11-dependent manner. Taken together, our data indicate that Dyskerin, and the H/ACA snoRNPs are essential for LSU biogenesis in humans. This is in contrast to yeast where they are essential for SSU production. Furthermore, we also speculate that DKC patients are likely to have defects in ribosome biogenesis which result in increased p53 levels.

637-B TDRD3 recruits FMRP and the topoisomerase TOP3 β to spliced mRNAs and provides a molecular link between schizophrenia and fragile X syndrome

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¹University of Wuerzburg; ²Center for Neurobehavioral Genetics, Semel Institute for Neuroscience and Human Behavior, UCLA, Los Angeles, California, USA; ³Pharma Research and Early Development, Roche Diagnostics GmbH, Germany; ⁴Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK

The fragile X mental retardation protein (FMRP) affected in fragile X syndrome is a key regulator of eukaryotic mRNA translation. However, how it is recruited into target mRNA-protein complexes (mRNPs) remains enigmatic. We have previously identified an interactor of FMRP, the Tudor domain containing protein TDRD3, whose binding is disrupted by the highly pathogenic I304N missense mutation of FMRP. TDRD3 also associates with the exon junction complex (EJC) and binds asymmetrically dimethylated arginines (aDMA) in histones and the C-terminal domain of RNA-polymerase II. Therefore, we reasoned that TDRD3 might function in the recruitment of FMRP to mRNPs. Here we report that FMRP and TDRD3 form a complex with a third component, the DNA topoisomerase TOP3 β . Interestingly, deletion of the gene encoding TOP3 β is associated with schizophrenia and intellectual disability. We provide evidence that this enzyme, previously implicated only in the unwinding of DNA, is a cytoplasmic RNA binding protein (RBP). Thus, the etiologies of schizophrenia and FXS might intersect at the post-transcriptional regulation of mRNPs containing TOP3 β and FMRP. Interestingly, formation of the TOP3 β -TDRD3-FMRP complex and its ability to recognize aDMA are essential for the concomitant integration of TOP3 β and FMRP into mRNPs. The biochemical composition of these mRNPs and their association with polyribosomes further suggests that they are engaged in the pioneer round of translation. Hence, our data uncover a novel mechanism for the recruitment of FMRP into mRNPs that is independent of RNA cis-elements and provide a molecular link between two neuropsychiatric disorders.

640-B Investigating the role and regulation of microRNA-10a in Acute Myeloid Leukaemia bearing the Nucleophosmin1 mutation

*Thi Thanh Vu*¹, *Catalina Palma*¹, *Adam Bryant*¹, *Dima Al Sheikha*¹, *Vivek jayaswal*², *David Ma*¹

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An increasing body of evidence has demonstrated the role of microRNAs in cancers. Acute Myeloid Leukemia (AML) associated with *Nucleophosmin1* gene mutation (*NPM1c+*) accounts for a third of AML cases (1). Our previous work has reported a unique microRNA signature in AML-*NPM1c+* samples which is dominated by 19.6-fold increase in hsa-miR-10a expression in the *NPM1c+* AML compared to wild type (wt) samples (2). We have demonstrated the role of miR-10a in apoptosis using human OCI-AML3 cell line, which is the only cell line known to harbour *NPM1c+*. The knock down of miR-10a results in an increase in cell death and a decrease in colony forming potential. These effects thought to be mediated through miR-10a targeting of *ARNT*, *GTFH1*, *ID4*, *KLF4*, *MAPRE1*, *NR4A3*, *RB1CC1* and *TFAP2C* (confirmed by luciferase assay), all of which are associated with neoplastic transformation. This suggests that miR-10a may act as a pro-survival factor *in vitro*. We aim to expand our study on miR-10a's leukaemic role in AML by knockdown of miR-10a in AML clinical samples. MiR-10a has been previously reported to be negatively regulated by methylation (3). To explore the mechanism that leads to the dysregulation of miR-10a in *NPM1c+*AML, we assess the role of methylation of the CpG islands, which is located within 2kb upstream of the miR-10a promoter. Mutations in *DNA methyltransferase*, *DNMT3A* has been frequently found in *NPM1c+*AML (4), leading to our investigation if *DNMT3A* mutations are associated with differential methylation patterns in *NPM1c+*AML, explaining the specificity of miR-10a overexpression in this AML subtype.

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643-B Endogenous shRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells with High Purity.*James Yin¹***¹The military general hospital of Beijing, and Institute of Biophysics**

Our preliminary studies in 2008 discovered 2564 new endogenous short hairpin RNAs (shRNAs) in human by bioinformatics and solexa sequencing, one (termed as shR-EID1 or shR-337) of which has been found to induce conversion of MSCs into HSCs by inhibiting the expression of EIA-like inhibitor of differentiation 1 (EID1). EID1 interacts with CREB binding protein (CBP) and p300, which have at least 400 interacting protein partners (25), and these proteins play a crucial role during hematopoiesis. Mesenchymal stem cells (MSCs) can differentiate into cells of bone, endothelium, adipose tissue, cartilage, muscle, and brain. However, whether they can transdetermine into hematopoietic stem cells (HSCs) remains unsolved.

We report here that human MSCs with CD44⁺, CD29⁺, CD105⁺, CD166⁺, CD133⁻, and CD34⁻ could differentiate into hematopoietic stem cells (CD150⁺/CD49f⁺/CD133⁺/CD34⁺) and their descending blood cells *in vitro*, when transfected with a new endogenous shRNA. The shRNA was high-effectively delivered into MSCs by a novel peptide means. These induced MSC-HSCs could form different types of hematopoietic colonies as nature-occurring HSCs did. Upon transplantation into sublethally irradiated NOD/SCID mice, these MSC-HSCs engrafted and differentiated into all hematopoietic lineages such as erythrocytes, lymphocytes, myelocytes and thrombocyte. Furthermore, we demonstrated the first evidence that the transdetermination of MSCs was induced by acetylation of histone proteins and activation of many transcriptional factors. These transfected MSCs could be converted into other types of stem cells in the presence of appropriate cytokines. Together, our findings identify the endogenous shRNA that dictates a directed differentiation of MSCs toward HSCs and open up a new source for HSCs used for the treatment of blood diseases and artificial stem cell-made blood.

Viral RNAs

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 646 B – 652 B

Location: Main Hallway & Sanada Foyer

646 B **Mechanistic insights into EMCV-IRES translation initiation**

649 B **Thermodynamics of HIV-1 Reverse Transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors**

652 B **Structural rearrangements of HIV-2 RNA during dimerization**

646-B Mechanistic insights into EMCV-IRES translation initiation*Nathalie Chamond¹, Jules Deforges¹, Nathalie Ulryck¹, Audrey Brossard², Bruno Sargueil¹*¹CNRS - Université Paris Descartes - UMR8015; ²CNRS - Université Paris Descartes - UMR8015

Most of eukaryotic mRNAs are translated by the cap-dependent pathway which relies on the recognition of the cap structure. This is mediated by the eIF4F complex which contains the eIF4A helicase and the eIF4G platform which coordinates 40S and mRNA association. Viruses have developed many ways to hijack their host translation machinery, among which, internal entry of the ribosome which allows a direct recruitment of the initiation complex near or at the AUG codon, without requiring a 5'-cap. This mechanism relies on RNA structures called IRESes (Internal Ribosome Entry Sites), generally present in the 5'-UTR. Although numerous viral IRESes have been identified, they differ from one another, leading to the hypothesis that many different mechanisms exist. Initiation of translation on type II IRESes such as those present in the Encephalomyocarditis Virus (EMCV) and in the Foot and Mouth Disease Virus (FMDV) recruit eIF4G/A which in the presence of ATP restructures the border of the IRES. These conformational changes are thought to allow the recruitment of 43S pre-initiation complexes *via* the eIF4G/eIF3 interaction. However, eIF4G mutants lacking the ability to contact eIF3 efficiently promote initiation on EMCV-IRES. This prompted us to evaluate a direct binding of the 40S subunit to EMCV-IRES. By using a combination of approaches we were able to demonstrate that EMCV-IRES directly binds the 40S subunit leading to a stable binary complex. Deletion mutants show that the region between the H and L domains is necessary for optimal 40S binding which is in agreement with their requirement for optimal IRES activity. This was further confirmed by footprinting analysis that identifies the H domain as part of the 40S binding site. In addition, as previous results on EMCV translation indicated that the nature of the downstream gene interferes with translation efficiency, we analyzed 40S binding from constructs presenting different open reading frames. Toe-print analysis of these RNAs in the presence of 40S subunits unravels a specific structure in the open reading frame that influences positioning of the mRNA in the 40S decoding groove. Taken together, these results allow us to refine the model for EMCV-IRES translation initiation.

649-B Thermodynamics of HIV-1 Reverse Transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors

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HIV-1 reverse transcriptase (RT) is a heterodimeric enzyme that converts the genomic viral RNA into proviral DNA. Despite intensive biochemical and structural studies, direct thermodynamic data regarding RT interactions with its substrates are still lacking. Here we addressed the mechanism of action of RT and of non-nucleoside RT inhibitors (NNRTIs) by isothermal titration calorimetry (ITC). Using a new incremental-ITC approach, a step-by-step thermodynamic dissection of the RT polymerization activity showed that most of the driving force for DNA synthesis is provided by initial dNTP binding. Significant differences are observed between nucleotide incorporation in elongation (RT bound to a DNA duplex) and in initiation (RT bound to the HIV-1 Primer Binding Site RNA/tRNA^{Lys}(3) duplex) of reverse-transcription. Surprisingly, thermodynamic and kinetic data led to a complete re-interpretation of the mechanism of inhibition of NNRTIs. Binding of NNRTIs to preformed RT/DNA or RNA complexes is hindered by a kinetic barrier and NNRTIs mostly interact with free RT. Once formed, RT/NNRTI complexes bind NA either in a seemingly polymerase-competent orientation, or form high-affinity dead-end complexes, both RT/NNRTI/DNA or /RNA complexes being unable to bind the incoming nucleotide substrate.

652-B Structural rearrangements of HIV-2 RNA during dimerization*Katarzyna Pachulska-Wieczorek¹, Katarzyna J. Purzycka¹, Ryszard W. Adamiak¹*¹RNA Structure and Function Laboratory, Institute of Bioorganic Chemistry, Polish Academy of Sciences

Human retroviruses HIV-1 and HIV-2 are characterized by similar genome organization, virion structure and replication cycle. Like other retroviruses, they possess dimeric RNA genome assembled from two identical sense strands, interacting near their 5'-ends. The full-length HIV-2 RNA serve both as genomic RNA which are packaged into nascent virions and as the template for translation of Gag and Gag-Pol precursor polyproteins. HIV-2 dimerization and encapsidation signals are linked and located in the leader RNA region (5' UTR). Dimerization is a regulated process that involves several RNA structural changes governed by the Gag and NC proteins.

In order to gain a deeper understanding of HIV-2 RNA dimerization and packaging, we applied chemoenzymatic probing to the in vitro assembled loose dimer. Our studies indicated that the dimeric structure is held by the direct interactions of the palindromic sequences within SL1 and TAR domains. Our combined structural and functional analysis allowed us to characterize previously unknown structural rearrangements and extensive tertiary interactions crucial for the mature dimer formation. It is presumed that in the case of HIV-2, cotranslational capture of the full-length RNA by nucleocapsid domain of Gag ensures encapsidation specificity. Our results on nucleocapsid protein binding provide new insight into mechanism of HIV-2 genomic RNA differentiation from other *gag* mRNA species.

Interconnections Between Gene Expression Processes

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 655 B – 670 B

Location: Main Hallway & Sanada Foyer

- 655 B** **Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes**
- 658 B** **SC35 and Promoter-Associated Nascent RNA Function like HIV Tat and TAR to Regulate Transcription Pause Release**
- 661 B** **The RNA exosome promotes a transcription termination pathway coupled to RNA decay**
- 664 B** **Traf3 alternative splicing during T cell activation: functional consequences and regulatory insights**
- 667 B** **Molecular mechanisms of RNA Polymerase II termination mediated by contacts with Rat1 and CF IA**
- 670 B** **Messenger RNA decay rates feedback to influence transcription and buffer gene expression in mammalian cells**

655-B Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes*Inna Afasizheva¹, Lan Huang², Ruslan Afasizhev¹***¹Department of Molecular & Cell Biology, Boston University Medical Campus, Boston, MA 02118, USA; ²Department of Physiology & Biophysics, School of Medicine, University of California-Irvine, Irvine, CA 92697, USA**

The majority of trypanosomal mitochondrial pre-mRNAs undergo massive uridine insertion/ deletion editing which creates open reading frames. However, our recent findings indicated that pre- and post-editing processing steps are also required to produce translation-competent mRNAs. Pre-editing addition of short 3' A-tails exerts no influence on unedited pre-mRNA stability, but stabilizes transcripts that are edited beyond few initial sites. The post-editing extension of A-tails into A/U heteropolymers by KPAP1 poly(A) polymerase and RET1 TUTase commits fully-edited mRNAs to translation. To identify factors responsible for coupling of editing, polyadenylation and translation we built a comprehensive protein interactions network of respective machineries. The ensuing RNAi screen distinguished several pentatricopeptide repeat-containing (PPR) RNA binding proteins acting to: 1) stabilize mRNA prior to polyadenylation (PPR15); 2) block premature mRNA uridylation (PPR14) and 3) induce transcript-specific adenylation/ uridylation (PPR26 and PPR28). PPR15 is similar to the kinetoplast polyadenylation/ uridylation factor 1 (KPAF1) and is an integral subunit of the polyadenylation complex. PPR14 associates transiently with both KPAP1 and RET1, and is likely to be a membrane-anchored protein. Unexpectedly, PPR26 and PPR28 are stably associated with small ribosomal subunit (SSU) and the polyadenylation complex. Investigation of these PPR proteins suggested that pre-mRNA is initially stabilized by PPR15 binding which may also recruit poly(A) polymerase resulting in A-tail addition. Initiation of RNA editing at the 3' region displaces PPR15 leaving A-tail as the main cis-acting stability element. We propose that SSU-associated PPR proteins, such as PPR26 and PPR28, recognize specific RNA sequences and stimulate transcript-selective A/U-tailing thereby committing mRNA for translation. Finally, PPR14 is likely to be actively disengaged from fully-edited mRNA to allow for A/U-tail addition.

658-B SC35 and Promoter-Associated Nascent RNA Function like HIV Tat and TAR to Regulate Transcription Pause Release*Xiong Ji¹, Xiang-Dong Fu²***¹Wuhan University, China; ²UC, San Diego**

Paused RNAP II at gene promoters produces short nascent RNA of unknown function, and the transition to productive elongation requires active recruitment of the transcription elongation factor P-TEFb. On the HIV-1 promoter, the virus-encoded protein Tat activates transcription elongation by relocating P-TEFb from the inhibitory 7SK complex to promoter proximally paused RNAP II via binding the TAR element in nascent viral RNA, but whether cellular genes use an analogous mechanism to activate transcription is unclear. We present evidence that SC35 (also known as SRSF2, a prototypical SR family splicing factor) has functions analogous to Tat, and that SC35 binding sites in nascent transcripts play roles equivalent to TAR in overcoming RNAP II pausing near the transcription start. These findings reveal an unanticipated SR protein function, a role for promoter-proximal nascent RNA at a discrete step in gene expression, and a unifying mechanism for transcription activation of viral and cellular genes.

661-B The RNA exosome promotes a transcription termination pathway coupled to RNA decay*Jean-Francois Lemay¹, Marc Larochelle¹, Samuel Marguerat², Jürg Bähler³, Francois Bachand¹*

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The transcription cycle is composed of three essential steps that consist of initiation, elongation, and termination. Termination is probably the least understood of these three steps in eukaryotic cells. For protein-coding genes, termination is normally initiated by the endonucleolytic cleavage of the nascent mRNA, which generates an entry point for a 5'-3' exonuclease-dependent termination pathway known as the torpedo model. In contrast to this 5'-3' termination pathway, we provide evidence for a new mechanism of transcription termination that depends on the 3'-5' exonuclease activity of the RNA exosome. Accordingly, transcriptome-wide analyses of fission yeast cells depleted for exosome subunits reveal widespread accumulation of 3'-extended transcripts from coding and noncoding genes, whereas mature RNA levels are not reduced. Importantly, the detection of read-through RNAs in exosome-deficient cells strongly correlates with a genome-wide increase in RNA polymerase II density at the 3' end of genes, consistent with transcription termination defects in the absence of a functional exosome. We show that RNA exosome subunits are present along transcribed genes and are recruited in a transcription-dependent manner, supporting a direct role for the exosome in promoting transcription termination. Our results also indicate that the exonucleolytic activity, but not the endonucleolytic function of the Dis3 catalytic subunit is required for exosome-dependent termination. These findings support a reverse torpedo model in which the 3'-5' exonucleolytic activity of the RNA exosome promotes the release of RNAPII and the concomitant degradation of the nascent transcript.

664-B Traf3 alternative splicing during T cell activation: functional consequences and regulatory insights*Monika Michel¹, Ilka Wilhelmi¹, Marco Preussner¹, Florian Heyd¹*¹**Philipps-University Marburg**

The non-canonical NFκB (ncNFκB) pathway regulates the expression of chemokines required for secondary lymphoid organ formation and maintenance and thus plays a pivotal role in adaptive immunity. ncNFκB activity is controlled by the kinase NIK, which itself is negatively regulated by the full-length isoform of Traf3. Here we show that T cell specific and activation-dependent alternative splicing generates a Traf3 isoform lacking exon 8 (Traf3DE8) that, in contrast to the full-length protein, activates ncNFκB signaling. Traf3DE8 disrupts the NIK-Traf3-Traf2 complex and allows accumulation of NIK to initiate ncNFκB signaling in activated T cells. ncNFκB activity results in the expression of several chemokines, among them CxCL13, both in a model T cell line and in primary human CD4⁺ T cells. As CxCL13 plays an important role in B cell migration and activation, our data suggest an involvement and provide a mechanistic basis for Traf3 alternative splicing and ncNFκB activation in regulating T cell dependent adaptive immunity.

To start investigating the mechanism of activation-induced Traf3 alternative splicing we have used minigene analysis and defined an intronic cis-regulatory element. RNA-protein interaction studies and an siRNA screen have yielded candidate trans-acting factors that we are now testing for their involvement in regulating Traf3 exon 8 exclusion in activated T cells.

667-B Molecular mechanisms of RNA Polymerase II termination mediated by contacts with Rat1 and CF IAErika Pearson¹, Claire Moore¹¹Tufts University School of Medicine

Proper RNA Polymerase II (Pol II) transcription termination is needed to generate stable transcripts, prevent transcriptional interference with downstream loci, and allow recycling of Pol II back to the promoter. Termination is tightly coordinated with 3' end RNA processing and is intricately regulated by a variety of different *cis*- and *trans*-acting factors. Although many eukaryotic termination factors have been identified, the precise molecular mechanisms governing this essential process remain to be elucidated. A major limitation in our understanding of termination has been the lack of a defined, easily manipulated *in vitro* system. We have thus developed and characterized an *in vitro* transcription system for the assay of factor-driven Pol II termination. In this system, we generate promoter-driven stalled elongation complexes from yeast whole cell extract and challenge these complexes with purified termination factors. We show for the first time that the exonuclease Rat1, in complex with its stabilizing partner Rai1, can elicit release of stalled Pol II *in vitro* and can do so in the absence of other termination factors. We also find that Rtt103, which interacts with the Pol II C-terminal domain (CTD) and with Rat1, can rescue termination activity of an exonucleolytic-deficient Rat1 mutant. In light of our findings, we posit a model whereby functional nucleolytic activity is not the feature of Rat1 that ultimately promotes termination. Degradation of the nascent transcript allows Rat1 to pursue Pol II in a guided fashion and to arrive at the site of RNA exit from Pol II. Upon this arrival, however, our model suggests that it is perhaps the specific and direct contact between Rat1 and Pol II that transmits the signal to terminate transcription.

To further explore the role of direct interactions between Pol II and termination factors, we have characterized a Pol II variant with a deletion of the flap domain, an evolutionarily conserved, surface-exposed region within the vicinity of the RNA exit channel. This Pol II variant is not defective for transcription elongation but exhibits defective termination of short RNAs *in vitro* and of snoRNAs *in vivo*. Additionally, the mutation significantly alters the *in vitro* and *in vivo* interactions between Pol II and Cleavage Factor IA (CF IA), a complex important for both 3' end processing and termination. These findings suggest that contacts of proteins with the body of Pol II, in addition to those formed with the CTD, are important for efficient termination.

670-B Messenger RNA decay rates feedback to influence transcription and buffer gene expression in mammalian cells

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There is considerable evidence that transcription influences mRNA decay and several models for how this can occur have been put forward. However, we believe that the cell may also have mechanisms by which mRNA decay rates can also feedback and influence transcription. This assertion is based on some recent results generated in our laboratories. First, we examined the differences in mRNA decay between human foreskin fibroblasts (HFF) and genetically identical induced pluripotent stem (iPS) cells that were derived from these HFFs. Not surprisingly, there were many differences in mRNA decay rates, but when we assessed changes in mRNA abundance between the two cells we were surprised to find that there was a negative correlation between decay and abundance. Transcripts that were more stable in iPS cells were frequently of lower abundance than in HFFs. Conversely, destabilized transcripts tended to be more abundant. Next, we examined the effects of perturbing mRNA decay on transcription. We undertook a global analysis of mRNA decay rates in C2C12 myoblasts following depletion of the deadenylase PARN. We determined that several mRNAs were significantly stabilized in a PARN knockdown cell line. Intriguingly, as before, we found that the abundance of these stabilized transcripts either was not altered, or was slightly reduced. The opposite was true for the transcripts that were destabilized in PARN KD cells – their abundance tended to increase. We believe that these destabilized mRNAs are affected through indirect mechanisms making the opposing effects on transcription even more surprising as they are unlikely to be mediated by effects of PARN on either transcription or mRNA decay. To investigate this phenomenon further, we validated the changes in mRNA decay and abundance for four of the stabilized transcripts by qRT-PCR. We then measured the abundance of newly transcribed pre-mRNAs as an indicator of transcription rate. In each case, despite clear decreases in mRNA decay rates, the mRNA abundance and pre-mRNA abundance was surprisingly reduced instead of increased as current models would predict. These results imply that the cell is able to buffer mRNA levels by down-regulating transcription to compensate for reduced mRNA decay.

RNA system biology

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 673 B – 679 B

Location: Main Hallway & Sanada Foyer

- 673 B** **Modeling the RNA-binding specificity of GLD-1 suggests a function of coding region-located sites in translational repression**
- 676 B** **Global gene regulation mediated by intron retention during T cell activation**
- 679 B** **A Potential Role for snoRNAs in PKR Activation during Metabolic Stress**

673-B Modeling the RNA-binding specificity of GLD-1 suggests a function of coding region-located sites in translational repression

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To understand the function of RNA-binding proteins (RBPs) encoded in animal genomes, it is important to identify their target RNAs. Although it is generally accepted that the binding specificity of an RBP is well described in terms of the nucleotide sequence of its binding sites, other factors such as the structural accessibility of binding sites or their clustering, to enable binding of RBP multimers, are also believed to play a role. Here we focus on GLD-1, a translational regulator in *Caenorhabditis elegans*, whose binding specificity and targets have been studied with a variety of methods such as CLIP (crosslinking and immunoprecipitation), RIP-Chip (microarray measurement of RNAs associated with an immunoprecipitated protein), profiling of polysome-associated mRNAs and biophysical determination of binding affinities of GLD-1 for short nucleotide sequences. The latter approach appears to allow an accurate reconstruction of the sequence specificity of the protein and may be applied to uncover the specificity and function of other RBPs. Taking into account the accessibility of putative target sites significantly improves the prediction of our GLD-1 binding model, in particular due to a more accurate prediction of binding in the transcript coding regions. Relating GLD-1 binding to translational repression and stabilization of its target transcripts we find that binding sites along the entire transcripts contribute to functional responses.

676-B Global gene regulation mediated by intron retention during T cell activation*Ting Ni¹, Wenjing Yang³, Weiqun Peng², Keji Zhao⁴, Jun Zhu¹***¹Genetics and Development Biology Center, National Heart Lung Blood Institute; ²Department of Physics, George Washington University; ³Genetics and Development Biology Center; ⁴system biology center, National Heart Lung Blood Institute**

Large-scale genomics studies have shown that gene activation and/or repression, as determined at the steady-state mRNA level, are often demarcated by corresponding changes in variant histone marks (e.g. H3K4me3, H3K9me3). Such concordance, however, is lacking for transcripts differentially expressed between resting and activated human CD4⁺ T cells, suggesting posttranscriptional gene regulation might be involved. To solve this conundrum, strand-specific RNA-seq was employed to profile T-cell transcriptomes before and after extracellular stimulation. Our results showed that resting T cells exhibit a higher than expected level of intronic tags, the proportion of which is significantly reduced upon T cell activation. Intron retention in fact is a widespread phenomenon as demonstrated by IRI (intron retention index) analysis, a newly devised approach to monitor intron retention at the individual gene level. In addition, by integrating a large collection of ChIP-seq data, we further demonstrated that intron-retained transcripts are unstable and possibly degraded by RNA surveillance complex in the nucleus. More strikingly, fold change in intron retention level is negatively correlated with that of steady-state mRNA level, indicating that global intron retention may serve as a major contributor for gene regulation during T cell activation. Supporting this notion, majority of the genes upregulated in activated T cells are accompanied with a significant reduction in intron retention. Of them, 267 genes are likely to be regulated solely at the posttranscriptional level, and are highly enriched in the pathways that are essential for proper T cell proliferation and cytokine release. Taken together, our study uncovered a novel posttranscriptional mechanism mediated by global intron retention. It can bypass the requirement for *de novo* transcript synthesis and therefore shorten the responding time to extracellular stimuli such as acute infection.

679-B A Potential Role for snoRNAs in PKR Activation during Metabolic Stress*Osama Youssef¹, Takahisa Nakamura³, Gökhan Hotamisligil², Brenda Bass¹*¹Department of Biochemistry, University of Utah, Salt Lake City, UT; ²Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA; ³Division of Endocrinology at Cincinnati Children's Hospital Medical Center, Cincinnati, OH

PKR (Protein Kinase RNA activated) is a member of the stress-response kinase family. Catalytically active PKR inhibits translation by phosphorylating the eukaryotic initiation factor, eIF2a. PKR contains a regulatory dsRNA-binding domain at its N-terminus and a catalytic kinase domain at its C-terminus.

PKR has long been known to be activated by viral dsRNA as part of the mammalian immune response. However, PKR kinase activity is also stimulated under conditions of metabolic stress in mice. This stimulation requires a functional kinase domain, as well as a functional dsRNA-binding domain. However, the cellular dsRNA required for PKR activation during metabolic stress is unknown. We investigated this question using Mouse Embryonic Fibroblast (MEF) cells expressing wildtype PKR (PKR_{WT}) or PKR with a point mutation in each dsRNA-binding motif (PKR_{RM}). Cells were incubated in the presence or absence of palmitic acid (PA), to mimic a high-fat or regular diet, respectively, followed by immunoprecipitation of PKR. PKR immunopurified RNAs from two different sets of three biological replicates were subjected to high-throughput sequencing. Since it is predicted that PKR_{RM} does not bind to RNA activator(s), RNAs enriched in both immunopurified PKR_{WT} and PKR_{RM} after PA treatment were excluded from our analysis.

We found that 122, and 90, exons were enriched by = 2-fold in PKR_{WT} samples after PA treatment (FDR ≤ 5%) in the first and second datasets, respectively. Interestingly, 43% and 78% of the enriched exons encode snoRNAs in the first and second datasets, respectively. An alternative bioinformatics approach using a sliding window across the genome showed that 224 regions were enriched in PKR_{WT} after PA treatment (Fold increase = 2, q-value FDR ≤ 5%). Of these, 112 were snoRNAs; the large majority of the remaining regions were annotated mRNA transcripts, but a few unannotated regions and lncRNAs were also observed.

Immunoprecipitation of PKR in extracts of UV-crosslinked cells, followed by RT-qPCR, was used to confirm that snoRNAs specifically associated with PKR_{WT} after PA treatment. These studies showed that snoRNAs were enriched in PKR_{WT} after PA treatment, but not in the PKR_{RM} samples.

CHO cells haploinsufficient for the spliceosomal protein SmD3 maintain pre-mRNA splicing, but show reduced levels of intronic snoRNAs¹. Consistent with the idea that snoRNAs trigger PKR activation after PA treatment, wildtype CHO cells, but not SmD3 haploinsufficient cells, showed increased PKR phosphorylation after PA treatment. Further, the decrease in PKR phosphorylation in mutant cells correlated with a decrease in JNK phosphorylation.

Using gel-shift assays we also find that PKR_{WT} but not PKR_{RM} binds directly to snoRNAs.

Our results support an unprecedented and unexpected model whereby snoRNAs play a role in the activation of PKR under metabolic stress.

¹Scruggs, Michel, Ory, Schaffer, 2012 MCB.

Bioinformatics

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 682 B – 697 B

Location: Main Hallway & Sanada Foyer

- 682 B** EXOME-WIDE COMPUTATIONAL PREDICTION OF EVOLUTIONARILY CONSERVED CIS-REGULATORY G-QUADRUPLEX MOTIFS
- 685 B** Terminator: a method for precise detection of ncRNA ends and terminal stem-loops using chimeric reads from RNA-seq
- 688 B** Stepwise co-evolution between bacterial CRP/FNR-type transcription factors and their transcriptional networks
- 691 B** IntEREst: Intron-Exon Retention Estimation using RNA-seq data
- 694 B** Analysis of structural and functional impact of UTR single nucleotide variants identified in the non-small cell lung cancer by RNA-sequencing
- 697 B** Segmentation of Proximal RNA Binding Sites from High-throughput Sequencing Data Using a Density-Based Clustering Approach

682-B EXOME-WIDE COMPUTATIONAL PREDICTION OF EVOLUTIONARILY CONSERVED CIS-REGULATORY G-QUADRUPLEX MOTIFS

Scott Frees¹, Camille Menendez², Matthew Crum², Paramjeet Bagga²

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G-quadruplex structures formed by guanine rich nucleic acids have been implicated in important biological processes, human disease, and as therapeutic targets. Recently there has been much interest in studying the potential roles of RNA G-quadruplexes as *cis*-regulatory elements in post-transcriptional gene expression. Due to the technical limitations of experimental approaches, genome-wide computational analyses are needed for large-scale studies of G-quadruplexes. However, most computational methods have difficulty validating their predictions without laborious wet laboratory testing. We have developed a new method to map evolutionarily conserved G-quadruplex motifs in nucleic acid sequences. Cross-species motif conservation provides evidence for their biological relevance. We have implemented our method to develop a web-based software application and an exome-wide database of G-quadruplex motifs conserved across species. We have used these tools to study homologous G-quadruplex motifs in the context of 5', 3'-UTRs (untranslated regions) and coding sections of aligned mRNA sequences.

Our database contains more than 400,000 predicted homologous G-quadruplexes in the exomes of *Homo sapiens*, *Mus musculus*, *Pan troglodytes*, *Canis lupus familiaris*, *Danio rerio*, *Caenorhabditis elegans*, and *Kluyveromyces lactis*, constituting over 90,000 total genes. Conserved G-quadruplexes were mapped in the 5'- and 3'-UTRs of a large variety of genes, including which are involved in apoptosis, brain development, epigenetics, cell proliferation, oncogenesis, and transcription. We are using the database to study G-quadruplex composition, as well as distribution in the untranslated and translated exome regions with a view to investigate their biological roles as *cis*-regulatory elements.

685-B Terminator: a method for precise detection of ncRNA ends and terminal stem-loops using chimeric reads from RNA-seq

Zhipeng Lu¹, Greg Matera¹

¹Department of Biology, UNC at Chapel Hill

Non-coding (nc)RNAs play important roles in many aspects of gene regulation, and other cellular processes. The functions of ncRNAs are determined by both primary sequence and secondary structure, which typically act via base-pairing with other nucleic acid targets, or interacting with proteins and small molecules. Recent years have seen the development of a variety of computational and experimental methods that characterize novel ncRNA sequence and structure. One of these methods, RNA-seq, has been widely used for its high sensitivity and versatility.

Within RNA-seq data sets, we have discovered that many of the unmappable reads are chimeric. That is, these reads consists of two parts, one from the 5' or 3' end of the RNA, the other from the internal part of the opposite strand. This phenomenon clearly suggests self-priming from, and ligation with, terminal stem-loops during cDNA library preparation. Using the chimeric reads from existing RNA-seq data, we developed a program, called Terminator, to precisely determine the ends of ncRNAs and provide support for the predicted terminal stem-loops.

To obtain chimeric reads, we first use a short read mapper capable of partial mapping (not end-to-end) to analyze the original RNA-seq data, and select the partial reads. We then map the unmappable parts to the vicinity of the mapped locations, on the opposite strand. Finally, reads with two parts mapped to opposite strands are used to construct an ncRNA sequence/structure map. The terminally mapped half-reads indicate terminal stem-loops, whereas the internally mapped parts indicate single-stranded regions.

We have tested our method in several species (including *Drosophila* and human) in datasets that contain self-primed ncRNA reads. We confirmed the terminal stem loops and ends of well-studied ncRNAs, and also refined the precise ends for many newly discovered ncRNAs.

The combination of fast short-read mappers (we used bowtie2) and rapid local mapping of normally unmappable fragments allows efficient processing of large numbers of datasets in a short time, and therefore increases the sensitivity of our method. Terminal stem-loops and internal single-stranded regions are common features of most snRNAs and snoRNAs, and many other types of ncRNAs. With the ever-increasing amount of publicly available RNA-seq datasets, Terminator will be a useful tool for studying ncRNAs.

688-B Stepwise co-evolution between bacterial CRP/FNR-type transcription factors and their transcriptional networks

Motomu Matsui¹, Masaru Tomita¹, Akio Kanai¹

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Transcription is a key system in all living organisms. Transcription factors (TFs), in particular, play a central regulatory role(s) in this system, although the evolutionary scenarios of TFs and their coevolution with their target genes are poorly understood. Determining the relationships between the TFs and their target genes from an evolutionary perspective should contribute to our understanding of not only the variety of transcription regulation systems but also the archaic transcriptional networks.

The CRP/FNR-type TFs are members of a well-characterized global TF family in bacteria and have two conserved domains: the N-terminal ligand-binding domain for small molecules (e.g., cAMP, NO, or O₂) and the C-terminal DNA-binding domain. Although the CRP/FNR-type TFs recognize very similar consensus DNA target sequences, they can regulate different sets of genes in response to environmental signals. To clarify the evolution of the CRP/FNR-type TFs throughout the bacterial kingdom, we undertook a comprehensive computational analysis of a large number of annotated CRP/FNR-type TFs and the corresponding bacterial genomes. Based on the amino acid sequence similarities among 1,455 annotated CRP/FNR-type TFs, spectral clustering classified the TFs into 12 representative groups, and stepwise clustering allowed us to propose a possible process of protein evolution. Although each cluster mainly consists of functionally distinct members (e.g., CRP, NTC, FLP, and FixK), FNR-related TFs are found in several groups and are distributed in a wide range of bacterial phyla in the sequence similarity network. This result suggests that the CRP/FNR-type TFs originated from an ancestral FNR protein, involved in nitrogen fixation. Furthermore, a phylogenetic profiling analysis showed that combinations of TFs and their target genes have fluctuated dynamically during bacterial evolution. A genome-wide analysis of TF-binding sites also suggested that the diversity of the transcriptional regulatory system was derived by the stepwise adaptation of TF-binding sites to the evolution of TFs.

We are now expanding the survey objects and analyzing the transcriptional networks using the whole set of known TFs (approximately 300 proteins) in *Escherichia coli*. These results will be discussed in this conference.

[1] Matsui M., Tomita M. and Kanai A. (2013) *Genome Biol. Evol.* 5(2), 267-282.

691-B IntEREst: Intron-Exon Retention Estimation using RNA-seq data*Ali Oghabian¹, Elina Niemelä¹, Mikko Frilander¹*¹**Institute of Biotechnology, University of Helsinki, Finland**

RNA sequencing is widely used today for studying gene expression analysis as well as identifying and quantifying various mRNA processing intermediates in the cells. To estimate the levels of the partially spliced or unspliced transcripts in a cell from RNA-seq data we have developed IntEREst (Intron-Exon Retention Estimation) pipeline in R. The IntEREst pipeline can be run in parallel to increase the performance.

In this study we employed IntEREst to investigate the global analysis of the nuclear processing of the unspliced U12-type introns by the exosome, a ribonuclease complex involved in RNA turnover using ABI's SOLiD 4 transcriptome sequencing data. The U12-type introns are a distinct subset of nuclear introns and constitute >0.5 % of all introns (~800 introns in humans). They are removed via a separate spliceosome (so called U12-dependent spliceosome) while the normal U2-type introns are removed by the U2-dependent spliceosome. Earlier studies have shown that the splicing rate of the U12-type introns in the nucleus is slower than that of the U2-type, suggesting that the U12-type introns can regulate the levels of their "host" mRNAs in the cell. Consistently, an elevated level of unspliced U12-type introns have been detected in the steady-state mRNA populations in various organisms. Here we investigated the hypothesis that due to slower splicing, the unspliced mRNAs containing U12-type introns in the nucleus are preferentially degraded by the exosome complex. Exosome is a ribonucleoprotein complex that participates in numerous RNA processing and trimming steps but also degrades RNAs that either aberrant or accumulate in wrong compartments. We compared the retention levels of the U12- and U2-type introns in nuclear and cytoplasmic fractions in control cells and in cells in which the exosome function has been disabled (e.g. knockdown of the Rrp41 and Dis3 subunits). Analyzing more than 100,000,000 paired reads (50 bps + 35 bps, with ~100-200 bps library) that were mapped to U12 intron containing genes, we discovered that exosome inactivation stabilizes unspliced U12-type introns as opposed to the U2-type introns. Moreover, the effects of Rrp41 and Dis3 knockdowns were not identical, suggesting different regulatory roles for the two subunits. Finally, we also confirm in genome-wide fashion that unspliced U12-type introns are indeed overrepresented as compared to U2-type introns.

694-B Analysis of structural and functional impact of UTR single nucleotide variants identified in the non-small cell lung cancer by RNA-sequencing

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Non-small cell lung cancer (NSCLC) is the most common form of lung cancer, with high prevalence of mutations in KRAS gene. Tumors harboring these mutations tend to be aggressive and resistant to chemotherapy. In a recent study, the transcriptome of the tumors cells harboring mutant KRAS and wild-type KRAS have been compared to identify the differentially expressed genes, alternative splicing and single nucleotide variations (SNVs). About 40% of the total SNVs (73,717) identified were mapped to the UTR regions of mRNAs, however, their effects on the UTR region have not been analyzed due to the lack of

comprehensive computational pipeline. It is known that the UTR regions of mRNA harbor sequence and structural motifs that are involved in the post-transcriptional regulations of gene expression. SNVs mapped to these regions may cause sequence or structural changes that can interfere with the functions of UTR. Thus, we designed a pipeline to predict the effect of SNVs on the secondary structure of UTR (using RNAsnp) as well as miRNA target sites within the UTR regions of mRNAs (using TargetScan, miRanda and Rsearch).

With our comprehensive computational pipeline, we predicted 492 out of 29,290 UTR SNVs have significant effect on the local RNA secondary structure of the UTR regions (corresponding to 429 genes). Of these 492 SNVs, 117 SNVs were present in 104 genes involved in cancer related pathway. On the other hand, 302 out of 29,290 UTR SNVs were predicted to cause changes in the miRNA target site within the UTRs of 289 genes. Of these 302 SNVs, 73 were present in 67 genes involved in cancer related pathway. On these 67 genes, 85 miRNAs were predicted to bind either in the wild- type or mutant. We found 54 out of these 85 miRNAs overlap with lung cancer associated miRNAs from PhenomiR database, while six miRNAs are associated specifically with NSCLC according to microRNA body map database.

697-B Segmentation of Proximal RNA Binding Sites from High-throughput Sequencing Data Using a Density-Based Clustering Approach*Bob Zimmermann¹, Renée Schroeder¹*¹**University of Vienna**

High-throughput sequencing has been widely used to find novel protein-binding RNAs, utilizing methods such as CLIP-Seq and Genomic SELEX. Typical analyses of the high-throughput data employ the use of a histogram of the aligned read count per base, the intensity of which indicates, for a specific region of an RNA, the underlying sequence's relative occurrence of binding events in the experiment. This technique can, however, obscure the precise locations of binding sites in close proximity to each other, which would be found when analyzing high-throughput data for enzymes whose activity require multiple RNA binding sites, as well as for overlapping distinct ncRNA loci derived from processing events. We present a density clustering approach based on the OPTICS algorithm which can detect any number of RNA binding sites indicated by the data without prior knowledge of the expected length or number of binding sites in the transcript. Combined with histogram analysis as post-processing, the algorithm can detect minimal-length proximal binding sites of RNAs binding yeast RNA Polymerase II derived from Genomic SELEX data. Band-shift assays show that output of the algorithm corresponds both to the correct length as well as the binding affinity. The algorithm represents a novel approach to processing read data and is fast enough to run to large data sets after alignment of the reads is performed. It can be applied to many types of high-throughput sequencing data to deconvolute proximal DNA and RNA binding sites encoded in the genome. A C implementation of the algorithm will be available at <http://alu.abc.univie.ac.at/hillfinder>

Emerging & High-throughput Techniques for RNA

Date: Friday, June 14, 20:00 - 22:30

Abstracts: 700 B – 715 B

Location: Main Hallway & Sanada Foyer

- 700 B** Engineered “restriction RNases” for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids
- 703 B** Genome-wide profiling of RNA secondary structure in planta
- 706 B** tRid, a method for eliminating tRNAs without sequence information of tRNAs
- 709 B** Cytoplasmic mRNA Capping and the Implications of Cap Homeostasis
- 712 B** Discovery of gene expression inhibitors using a high throughput single-cell analysis system
- 715 B** A new technique for live cell RNA detection that enables single cell analysis and live cell sorting

700-B Engineered "restriction RNases" for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids

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¹Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Ul. Ks. Trojdena 4, 02-109 Warsaw, Poland.; ²Laboratory of Protein Structure, International Institute of Molecular and Cell Biology in Warsaw, Ul. Ks. Trojdena 4, 02-109 Warsaw, Poland.

Ribonucleases (RNases) are valuable tools applied in the analysis of RNA sequence, structure and function. Their substrate specificity is limited to recognition of single bases or distinct secondary structures in the substrate. Thus far, there have been no RNases available for purely sequence-dependent fragmentation of RNA, analogous to restriction enzymes for DNA. We have therefore searched for existing RNases that could be engineered to become sequence-specific. Using a combination of bioinformatics methods and experimental protein engineering we have obtained prototypes of two sequence-specific "restriction RNases" (RRNases): one that cleaves both strands of dsRNA within a target sequence, and one that cleaves RNA within DNA-RNA hybrids at a particular distance from the target sequence.

Based on structural analysis of enzymes from the RNase III superfamily we identified loops that could be extended to make specific contacts with bases in the dsRNA substrate. Biochemical characterization of selected members that possess extended versions of such loops revealed that some of them indeed exhibit sequence specificity. For one of such enzymes we constructed a structural model of a protein-RNA complex, and used it to guide site-directed mutagenesis aimed at elucidating the molecular basis of specificity and to increase the selectivity of cleavage. The obtained prototype RRNase recognizes a partially degenerated hexanucleotide target sequence and is capable of cleaving individual sites in long dsRNA molecules.

A prototype RRNase that cleaves the RNA strand in DNA-RNA hybrids 5 nucleotides from a nonanucleotide recognition sequence was constructed by fusing two functionally distinct domains: a non-specific RNase HI and a zinc finger that recognizes a sequence in DNA-RNA hybrids. The optimization of the fusion enzyme's specificity was guided by a structural model of the protein-substrate complex and involved a number of steps, including site-directed mutagenesis of the RNase moiety and optimization of the interdomain linker length.

For both types of RRNases we implemented methods of specificity engineering, to enable generation of variants specific for other target sequences, making it feasible to acquire a library of enzymes that recognize and cleave a variety of sequences, much like the commercially available assortment of restriction enzymes. Potentially, RRNases may be used *in vitro* for production of RNA molecules with defined length and termini, which may be a cheaper alternative to chemical synthesis; they may be also used *in vivo* for targeted RNA degradation.

703-B Genome-wide profiling of RNA secondary structure in planta*Yiliang Ding¹, Chun Kit Kwok¹, Yin Tang¹, Philip Bevilacqua¹, Sarah Assmann¹*¹**Pennsylvania State University**

RNA structure plays critical roles in regulating various post-transcription events involved in translation, splicing, and polyadenylation. However, lack of genome-wide *in vivo* RNA structural data limits our understanding of how RNAs fold and regulate gene expression *in vivo*. Herein we develop a platform to probe RNA structures on a genome-wide scale *in vivo*. With the application of next generation sequencing, we establish high-throughput *in vivo* RNA secondary structure profiling in *Arabidopsis thaliana*, a model plant species and modern eukaryote. This platform probes the RNA secondary structures of more than 10,000 transcripts, provides even coverage of each transcript, and gives excellent agreement on rRNA structures as a benchmark. This platform provides an accurate and quantitative genome-wide RNA structural map *in vivo* that reveals native RNA structural features that relate to numerous biological processes including translation initiation and regulation, alternative polyadenylation, and alternative splicing. This platform can be applied to different organisms, different tissues and different treatments which will allow the role of RNA structures in gene regulation to be understood on a genome-wide scale.

706-B tRid, a method for eliminating tRNAs without sequence information of tRNAs

Kazuki Futai¹, Hiroaki Suga²¹Department of Chemistry, Graduate School of Science, The University of Tokyo (Present affiliation: Life Science Center of Tsukuba Advanced Research Alliance, University of Tsukuba)

We here report a unique method, designated as tRid, for eliminating tremendous amount of tRNAs from any organism's RNA without knowing the sequence information of tRNAs. The key component of tRid is a flexible tRNA-acylation ribozyme, known as flexizyme. This catalyst is able to recognize the 3'-end CCA of tRNA, which is common sequence to all tRNAs used in organisms, and adds any one of a diverse repertoire of natural/non-natural amino acids to the 3'-OH of tRNA. Therefore, selective removal of tRNA would be possible through the aminoacylation using a biotinylated amino acid (Fig. 1).

For demonstrating tRid, HeLa S3 and *E. coli* <200 nt RNAs were treated by modified flexizyme, whose 3'-end nucleotide is oxidatively cleaved by sodium periodate for suppressing self-contamination of flexizyme (Fig. 2). After the treatment, 70-100 nt RNAs were purified and sequenced. As a result, the ratio of tRNAs derived from HeLa S3 and *E. coli* were decreased from 89% to 35% and 85% to 30%, respectively. This result means that tRid does not need the information of tRNA sequences so that it can eliminate tRNAs from any kinds of organism's RNA. For application of tRid, we tried to discover novel tRNA-sized RNAs from 70-100 nt RNAs treated by tRid. The ratio of tRNA-sized RNAs derived from HeLa S3 and *E. coli* were increased from 11% to 65% and 15% to 70%, respectively. We discovered several novel sRNAs from the enriched *E. coli* tRNA-sized RNAs and elucidated that these sRNAs are transcribed as similar size of tRNA by northern blotting. This result demonstrates tRid facilitates the efficient exploration of novel tRNA-sized RNAs. For another application of tRid, we purified <200 nt RNAs from pond water, which contains various kinds of microorganisms, and eliminated tRNAs by tRid. As a result, the ratio of tRNAs was decreased from 90% to 46% and the ratio of tRNA-sized RNAs was increased from 10% to 54%. This result means that tRid can be applied to metatranscriptome analysis.

In conclusion, tRid is a general-purpose method, which can eliminate any organism's tRNAs without the sequence information of tRNAs and can be applied various studies that is difficult to do because of the tremendous amounts of tRNAs.

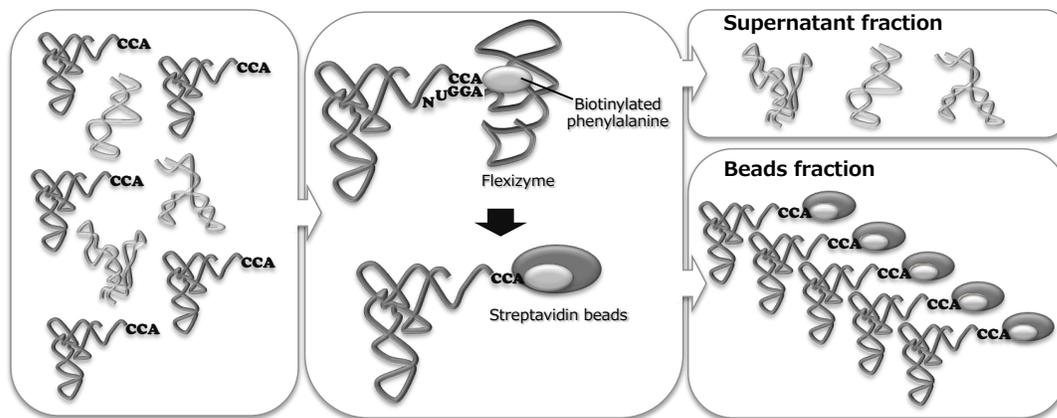


Fig. 1 Schematic illustration of tRid

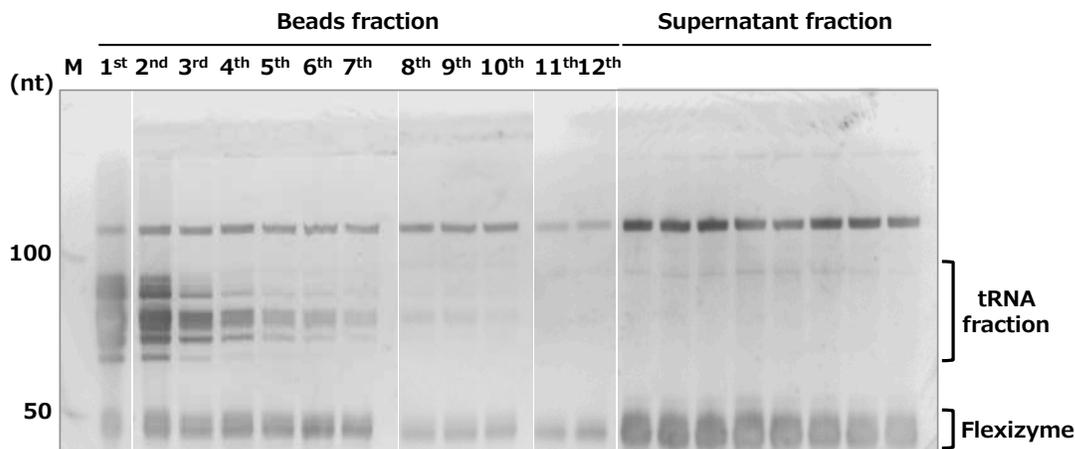


Fig. 2 The demonstration of tRNA elimination from <200 nt RNAs by tRid. *E. Coli* <200 nt RNAs were treated by flexizyme, biotinylated phenylalanine and streptavidin beads twelve times.

709-B Cytoplasmic mRNA Capping and the Implications of Cap Homeostasis*Daniel Kiss¹, Ralf Bundschuh², Daniel Schoenberg¹*¹**Department of Molecular and Cellular Biochemistry, The Center for RNA Biology, The Ohio State University;**²**Department of Physics, The Ohio State University**

Until recently, the removal of an mRNA's 5' cap structure was thought to be an irreversible step leading to the degradation of the decapped mRNA. Our lab has discovered and begun to characterize the cytoplasmic capping enzyme (cCE) complex, which restores the 5' cap onto certain decapped mRNAs. Results of experiments aimed at identifying recapping targets led to the discovery of cap homeostasis, a process by which some mRNAs undergo cyclical decapping followed by recapping. The regulated decapping and recapping of these mRNAs may function as mechanism for cells to survey and regulate their translation. Our previous work described the development of cells that are stably transfected with a tetracycline-inducible dominant negative form of cCE. Expressing dominant negative cCE increased the population of uncapped transcripts, and these were shifted from polysomes to non-translating mRNPs. Importantly, this is specific to recapping targets as the translation of control transcripts was unchanged. Interestingly, 5' RACE experiments performed with RNA from cells inhibited for cytoplasmic capping identified transcripts that were missing portions of their 5' ends, raising the possibility that cytoplasmic capping may enable the translation of N-terminally truncated proteins as well as full-length proteins from the same parent transcript. We couple informatics approaches with 5' RACE and sequencing to assess the diversity of these validated cCE targets and to pinpoint the locations where these transcripts are recapped. Finally, we are adapting ribosome profiling for use in our dominant negative cCE cell line as a way to ascertain the prevalence and functional effects of cap homeostasis on a transcriptomic level.

Supported by NIH grant GM084177 to DRS. DLK is supported by a Pelotonia postdoctoral fellowship and by NIH grant T32 CA0093338-34.

712-B Discovery of gene expression inhibitors using a high throughput single-cell analysis system*Scott Stevens¹, Matthew Sorenson¹*¹**University of Texas at Austin**

The many macromolecular machines involved in eukaryotic gene expression have been individually studied in model organisms for decades. These efforts have been enhanced by the use of specific reporter genes that are sensitive to defects in an individual process. In recent years, it has become increasingly evident that gene expression processes in eukaryotes involve communication and coordination between all of the gene expression machineries. We have previously described the development of a versatile gene expression reporter for budding yeast that employs high-throughput flow cytometry. Cells harboring the reporter generate green and red fluorescence from spliced and unspliced transcripts respectively. Our reporter exhibits a unique signature for defects in many gene expression processes including transcription, pre-mRNA splicing, NMD, mRNA export and mRNA decay. Using a high throughput approach we have genetically explored contributions of knockout mutations to gene expression.

Many of the pathways in gene expression are targets for therapeutics and naturally occurring small molecules have provided a wealth of information about those pathways. Further work to identify novel targets and effectors will enhance our understanding of the control of gene expression and, importantly, provide potential new drug candidates. We have adapted our reporter assay to perform high-throughput screening for small molecule inhibitors of specific gene expression processes using an inducible version of our reporter. In our pilot study, we screened hundreds of small molecules that have a history of use in human clinical trials. We have followed these experiments with an expanded library of thousands of small molecules. By means of plate reader and flow cytometry analysis, we have identified many small molecules that modulate gene expression and affect cell growth. We are exploring the nature of the putative inhibition by these compounds which behave like pre-mRNA splicing, RNA decay or NMD inhibitors in our assay. Additionally, by virtue of the single-cell nature of our assay, we have identified molecules that increase cell-to-cell variation in reporter expression. We are currently identifying patterns between our primary hit compounds and the effect on eukaryotic gene expression pathways.

715-B A new technique for live cell RNA detection that enables single cell analysis and live cell sorting

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¹EMD Millipore

Understanding gene expression provides a window into the regulation of all cellular activity. To better understand and measure gene expression, various methods have been developed. Traditional techniques require long and laborious sample preparations and are prone to potential amplification bias. Other techniques involve transfections, cell fixation and cell lysis which could introduce unwanted change in expression levels.

A method for performing RNA detection in live cells would provide more biologically relevant information. Our technique allows for single-cell detection to accurately measure gene expression levels of living cells without stressful or harmful treatments. Because this technique does not involve harsh chemicals or transfection procedures, the data collected more accurately reflects a natural cell state. Furthermore following detection the cells remain unchanged and available for downstream assays.

This technique utilizes a gold nanoparticle surrounded by a high density oligonucleotide target specific sequence hybridized to complementary probes. Once in the cell, the gold nanoparticle will detect its target and release the fluorescent probe. The unquenched probe can then be accurately measured by flow cytometry for single cell RNA detection.

Here we show experiments using this technique for detection of RNA in single cells and live cell sorting by FACS with correlating qPCR data. In addition we prove this technique does not alter gene expression or affect cell health.