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Non-coding and Regulatory RNAs

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158-C Defense against viral attack: single-molecule view on a bacterial adaptive immune system

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Escherichia coli maintain different strategies to protect the cell against invading foreign DNA. In a recently discovered adaptive immune system, fragments of foreign DNA are integrated into specific loci on the bacterial genome, known as clustered regularly interspaced short palindromic repeats (CRISPR). Short CRISPR-derived RNAs (crRNAs) are incorporated into the CRISPR-associated complex for antiviral defence (Cascade) and guide the complex's search for the DNA of returning invaders, which is targeted for destruction upon binding. Recent studies have shown that Cascade must recognize both a short "seed" sequence and an immediately adjacent PAM (protospacer adjacent motif) sequence in order for successful targeting of the foreign DNA. The mechanism and structural dynamics of this target recognition and binding process, however, are not well understood. Here we report a single-molecule FRET (F?rster resonance energy transfer) based assay to monitor in real time the target recognition and binding process of Cascade. We find that there is directionality to the base-pairing process between the crRNA and the target DNA. Upon binding, pairing occurs first over the seed region and then, after a time lag, proceeds through a region further downstream on the target sequence. This suggests a mechanism for target recognition wherein the seed/PAM region is identified first, followed by a distinct kinetic intermediate, perhaps a structural transition, before subsequent target pairing can occur. Our single-molecule study promises to reveal the mechanism of target DNA identification by the CRISPR immune system.

161-C The role of RNA degradation in moderating RNAi

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Endogenous antisense non-coding RNAs could theoretically hybridise to their cognate sense RNA, forming double-stranded RNAs that are substrates for the Dicer endonuclease. Therefore, antisense transcripts have the potential to trigger RNA interference responses (RNAi) that would lead to the degradation of both sense and antisense RNA from a locus, providing a potent negative regulatory capability. Although antisense RNAs have been characterised at many loci in many organisms, they are generally unstable which would limit entry of sense-antisense pairs into the RNAi system. Antisense RNAs are susceptible to various degradation pathways acting in both the nucleus and cytoplasm, raising the potential for wide ranging gene expression regulation through control of antisense degradation.

S. cerevisiae does not possess an endogenous RNAi system, but such a system can be reconstituted by introducing Argonaute and Dicer from a related yeast species to form an RNAi+ strain [1]. We have tested the efficiency of RNAi in these RNAi+ cells for a number of sense-antisense systems in which the antisense RNA is unstable. Stabilisation of the *MAL32* antisense RNA by nuclear RNA degradation mutants increases the level of siRNAs produced from this locus, showing that stabilisation of antisense RNA in the nucleus can alter double stranded RNA formation as expected. However, altering cytoplasmic RNA levels by deletion of *XRN1* has more subtle effects; although levels of *GAL4* sense and antisense are increased in this mutant, there is no increase in siRNA production. In contrast, stabilisation of the *PDR3* antisense in the same cells leads to complete degradation of sense and antisense RNA by RNAi as predicted.

Our data show that RNA degradation can alter the susceptibility of mRNA to degradation by RNAi. This suggests a previously unrecognised role for the RNA degradation machinery in regulating entry of RNA from coding loci into the RNAi system. We are currently using high-throughput sequencing to elucidate genome-wide the effects of RNA degradation mutants on RNAi.

[1]: Drinnenberg, I. A. *et al*. RNAi in budding yeast. *Science* 326, 544-550 (2009).

164-C The exosome subunit Rrp6 regulates the expression of retrotransposons and non-coding heterochromatic sequences in Drosophila melanogaster

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The exosome is a multiprotein complex with ribonuclease activity that is highly conserved from archaea to eukaryotes. The exosome has many functions and acts on many different transcripts, but the mechanisms by which the exosome is recruited to its different substrates are not fully understood. We have applied immnoprecipitation and mass spectrometry to identify proteins associated with individual exosome subunits in *Drosophila melanogaster* S2 cells. Among the many proteins associated with the catalytic subunit Rrp6, we have found two functionally related factors involved in heterochromatin formation: the histone deacetylase Rpd3 and the histone methyltransferase specific for H3-Lys9, SuVar3-9. We have also shown that the Heterochromatin Protein 1, HP1a, is associated with Rrp6. Moreover, immunofluorescent staining of salivary gland polytene chromosomes revealed that Rrp6 is associated with heterochromatic regions of the genome. Knock-down of Rrp6 by RNA interference in *Drosophila* S2 cells resulted in increased levels of both transposon transcripts and non-coding RNAs originated from heterochromatic regions of the genome, whereas overexpression of Rrp6 had the opposite effect. Moreover, reduced levels of Rrp6 rendered the heterochromatin less compact, as shown by experiments of micrococcal nuclease digestion. Ongoing research aimed at understanding the function of Rrp6 in heterochromatin regulation will be presented.

167-C The RNA subunit of RNase MRP: extra nucleotides at the 3’ end

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RNase MRP is a eukaryotic ribonucleoprotein complex with specific endoribonucleolytic activity targeting a variety of vital cellular RNAs. A recent publication proposed a mechanism for regulation of the RNA subunit of MRP whereby RMRP RNA is a substrate for RNA dependent RNA polymerase activity when complexed with the protein subunit of human telomerase (hTERT)[1]. Our follow-up experiments demonstrate that, contrary to the previous report, the hTERT active site is not required for extension of RMRP. Instead, 3' extension activity is sensitive to alpha-amanitin and can be observed *in vitro* with purified RNA polymerase II. Further, traditional and next generation sequencing of RMRP 3' ends from total RNA of human cells reveals a distribution of terminal extensions from the annotated 3'-most nucleotide, including non-genomically encoded sequences. Sequencing of 3' ends of other common cellular RNAs yields somewhat more homogeneous profiles, notably lacking termini with complex non-genomically encoded sequences. Our current work aims to critically examine whether the *in vitro* additions recapitulate these produced *in vivo*, and ultimately characterize the significance of those extensions on RMRP function.

Reference

1. Maida, Y. *et al.* An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature* **461**, 230-235, doi:10.1038/nature08283 (2009).

170-C Identification of stage specific microRNAs during the developmental stages in Triops cancriformis (tadpole shrimp)

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microRNAs (miRNAs) are approximately 22 nucleotide non-coding RNAs that regulate gene expression at the post-transcriptional level, and play important roles in the cellular processes such as development and cell differentiation. Although many miRNAs are identified and analyzed in various model species during their development, it is unclear whether the miRNA functions are conserved among species or not. Here we focused on non-traditional model species *Triops cancriformis*, which is known as a "living fossil". *T. cancriformis* changes their morphology dramatically during the early larval developmetal stages. It was hypothesized that miRNA expression also dramatically changes in accordance with the morphological changes.

We first constructed small RNA libraries from six different stages of *T. cancriformis* development (egg, 1st to 4th instar larvae and adult). Deep sequencing analysis of these libraries resulted approximately 47 million reads. In parallel, we also performed deep sequencing analysis on genomic DNA that provided approximately 133 million reads. Bioinformatics analysis have shown 73 conserved miRNA sequences in *T. cancriformis* by using known miRNAs in other species registered in miRBase. It was confirmed that these 73 candidates were encoded on genomic DNA sequences and able to form a secondary structure of precursor miRNAs. Among these miRNA candidates, the expressions of eight *T. cancriformis* miRNAs were detected by northern blotting analysis among six developmental stages. We compared miRNA expression patterns of *T. cancriformis* with those of *D. melanogaster* reported from previous studies. As a result, *T. cancriformis* tcf-let-7 showed conserved expression profiles, whereas tcf-miR-87 showed different patterns. While tcf-miR-87 is strongly expressed in adult stage, *D. melanogaster* dme-miR-87 is detected in egg, 1st instar, 2nd instar, pupa, and adult stages. The inconsistency in the expression profile of conserved miRNAs suggest that these miRNAs may play different roles during development in each species, although they possess very similar sequences. The relationships between dynamic morphological changes and the miRNA expression pattern during the development will be discussed.

173-C A Natural Antisense Transcript is Involved in the Destabilization of Cyclooxygenase 2 mRNA by Acetaminophen

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Acetaminophen is a popular antipyretic and analgesic drug. Acetaminophen has a low incidence of adverse effects, when compared with non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase 2 (COX-2), the key enzyme in the biosynthesis of the inflammatory mediator prostaglandin. Because the mechanisms of action of acetaminophen are not fully understood, we sought to clarify the mechanism how acetaminophen affected the COX-2 expression. Similar to aspirin, a typical NSAID, acetaminophen reduced the levels of COX-2 protein and its mRNA in mouse macrophages treated with lipopolysaccharide (LPS). Acetaminophen suppressed the promoter activity of the *Cox-2* gene in the LPS-treated macrophages. We found that a natural antisense transcript (asRNA) was transcribed from the *Cox-2* gene in response to LPS, similar to the gene for inducible nitric oxide synthase, which produces another inflammatory mediator nitric oxide. The overexpression of COX-2 asRNA increased the expression of COX-2 mRNA. When we examined whether COX-2 asRNA modified the stability of COX-2 mRNA by reporter gene assays, acetaminophen canceled the effect of COX-2 asRNA and destabilized COX-2 mRNA. Our data suggest a possibility that COX-2 asRNA is a novel target of acetaminophen and a potent therapeutic target.

176-C Evf2 (Dlx6AS) long non-coding RNA regulation of interneuron gene expression and behavior

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The role of anti-sense (AS) gene regulation in controlling neural circuits and complex behavior are poorly understood. We previously showed that *Evf2 (Dlx6AS)* long non-coding RNA (lncRNA) represses Dlx6 in embryonic brain, affecting circuitry in adult hippocampus. However, behavioral effects of *Evf2* loss in mice are not known. A battery of behavioral tests reveals that mice lacking *Evf2* have reduced immobility in the forced swim test (FST), showing altered response to acute stress. Evf2 mutant mice behave normally in learning, motor, anxiety, and social interaction tests. Interneuron genetic fate-mapping analysis indicates significant loss of interneurons in Evf2 mutant adult brain. However, calcium binding protein expression is dramatically increased in remaining Evf2 mutant interneurons. Thus, elevated calcium binding proteins in Evf2 mutants are likely to be responsible for normal responses in many of the behavioral assays, as well as reduced immobility in FST. Truncation of *Evf2* that retains anti-sense Dlx6 transcription does not affect Dlx6 expression or FST immobility times. Together, these data indicate that loss of *Evf2(Dlx6AS*) anti-sense gene regulation can affect interneuron number and subtype, with behavioral consequences in adult mice.

179-C Molecular Mechanism Involved In Antisense-Mediated Transcriptional PHO84 Gene Silencing

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The development of new technologies such as tiling arrays and RNA-seq has revealed that eukaryotic genomes are extensively transcribed In *Saccharomyces cerevisiae* a class of non-coding transcripts produced by RNA pol II, called "cryptic unstable transcripts" (CUTs), is stabilized upon deletion of the nuclear exosome subunit Rrp6[(1)]. Whether this broad and unstable transcriptome has a regulatory role in gene expression is still unclear.

It has been previously shown that the *PHO84* gene is transcriptionally regulated by an antisense RNA that accumulates upon deletion of Rrp6, causing the recruitment of the histone deacetylase complex Hda1/2/3 which leads to the transcriptional repression of the *PHO84* gene[(2)].

A genetic screen aimed at further characterizing this mechanism has identified new factors that could be involved in antisense RNA-mediated silencing of the *PHO84* gene. Among those is the histone chaperone complex Hir, originally described as a key factor in the regulation of the histone gene expression during the cell cycle. However, more recent data support the idea of a possible global requirement of this complex in chromatin remodeling [(3)]. Our aim is to better characterize the role of this complex in the regulation of the *PHO84* gene in the absence of Rrp6. We hypothesize that antisense RNA production may facilitate the recruitment of the Hir complex to the *PHO84* gene promoter and contribute to its silencing.

[1] Neil et al., *Nature*, (2009):1038-1042

[2] J.Camblong, et al., *Cell*. (2007):706-717

[3] Fillingham J,et al., *Mol. Cell*. (2009): 340-51

182-C Revealing the elusive molecular biology of the vault RNA

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Recently several novel and previously reported non-protein-coding RNAs (ncRNAs) have been identified to be upregulated upon Epstein-Barr virus (EBV) infection in human B-lymphocytes. A group of these significantly upregulated ncRNAs are called vault RNAs (vtRNAs).[a,b] Only about 5% of the total cellular vtRNAs are connected to the vault particle, the largest known ribonucleoprotein particle (RNP) in eukaryotic cells. However the function of this ncRNA family and moreover of the vault particle remains still rather unclear. Our previous findings suggest a link between EBV infection and vtRNA expression. Consequently we are interested which part of the viral genome is responsible for the upregulation and moreover which function the vtRNAs might possess during virus propagation.

To address this question we have separately overexpressed specific EBV-encoded, latently expressed proteins in BL2-cells to determine the influence on the vault RNA levels. Thereby we identified one EBV-encoded protein, called Latent Membrane Protein 1 (LMP1), which significantly contributes to the vtRNA upregulation. We used LMP1 mutants to characterize the region of the protein and the responsible pathway for triggering the elevated vtRNA expression. Our results suggest that the NFkB- pathway might be involved in this process. To investigate a possible functional connection between the vtRNA and EBV infection, we have overexpressed vtRNA1-1 in BL41, a cell line usually not expressing this vault RNA. We show that overexpression of vtRNA1-1 leads to a better viral establishment and markedly protects cells from undergoing apoptosis. Knock-down of the major vault protein, the main component of the vault particle, had no effect on EBV infection and apoptosis resistance. Thus these results support the view that the observed phenotype is caused by the vtRNA rather than the vault particle.

[a] Mrázek, J., Kreutmayer, S.B., Grässer, F.A., Polacek, N., Hüttenhofer, A. (2007) Substrative hybridization identifies novel differentially expressed ncRNA species in EBV-infected human B cells. *Nucleic Acids Res*. 35,e73.

[b] Nandy C., Mrázek, J., Stoiber H., Grässer F.A., Hüttenhofer A., Polacek N. (2009) Epstein-barr virus- induced expression of a novel human vault RNA. J Mol Biol. 388 (4)

185-C Identification and functional characterization of the long non-coding RNA in myogenesis

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Eukaryotic genomes express a diverse range of non-coding RNAs as well as protein-coding mRNAs. Among them, some of long non-coding RNAs are known to have functions to regulate diverse biological processes, such as epigenetic control of chromatin, X-chromosome inactivation, and nuclear body formation. However, comparing to the large portion of long non-coding RNAs in entire genome, their functions remain largely uncharacterized. In particular, the potential contribution of long non-coding RNAs to the cellular differentiation is rarely elucidated. Myogenesis, the muscle differentiation, is a multistep process during which pluripotent mesodermal cells give rise to myoblasts that eventually differentiate into myotubes. During the course of myogenic differentiation, many muscle genes are up-regulated including MyoD, Myogenin, and MCK. Here, we took the advantage of genome-wide transcriptome analysis and found that the expression of not only muscle genes but many long non-coding RNAs is changed during myogenesis. There were many non-coding RNAs which are up-or down-regulated during muscle differentiation. Among the non-coding RNAs, we show the long non-coding RNA transcript, UT-10, which is increased during myogenesis has a potential role in muscle differentiation by regulation of muscle genes

188-C Mutations in the 5'UTR of SERPINA1 transcripts are involved in the disease associated mechanisms

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Single Nucleotide Polymorphisms (SNPs) are found throughout the human genome. SNPs implicated in disease-associated mechanisms that map to coding regions of the genome generally alter the function of the protein. However, disease-associated SNPs that map to non-coding regions can affect the translation regulation and/or stability of the mRNA. Novel high-throughput mappings of RNA binding protein sites found that most UnTranslated Regions (UTR) of mRNA are often targeted by proteins involved in the regulation of translation, stability of mRNA, and mRNA localization. Not only that many RNA binding proteins recognize specific primary sequences of the mRNA, but is also now well established that the secondary structure plays a critical role in the accessibility of a binding site. Thus, SNPs that significantly alter the structure of UTRs could affect post-transcriptional regulation.

Alpha 1-antitrypsin deficiency (A1AD) is an autosomal recessive genetic disorder caused by the defective production of alpha 1-antitrypsin (A1AT), a protein encoded by *SERPINA1*. Severe deficiency of A1AT causes panacinar emphysema or Chronic Obstructive Pulmonary Disease (COPD) as well as various liver diseases. It has been shown that certain mutations (Glu342Lys) in the coding regions of *SERPINA1* led to severe cases of COPD. A recent genome wide-association study identified a SNP in the 5' UTR of SERPINA1 associated with increased risk of developing COPD. SHAPE structure mapping analysis reveals that this SNP alters RNA structure acting forming a RiboSNitch – a SNP that induces a large conformational change of RNA resulting in an altered function of RNA. Thus, SNP induced RNA structure change likely plays an important role in COPD predisposition.

191-C Identification and characterization of rice non-coding RNAs involved in nitrogen-starvation stress response

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MicroRNAs and long non-coding RNAs are key regulators that control the expression level of genes by transcriptional and post-transcriptional regulation in animals and plants. Both of the non-coding RNAs are also involved in regulating plants' responses to stress conditions. Recent studies showed that non-coding RNA pool changes in some environmental conditions, implying that non-coding RNAs play key roles in responding to stress condition in plants. In this study, we aim to identify and characterize microRNAs and long non-coding RNAs induced by nitrogen-starvation stress in *Oryza sativa*. To do this, we performed RNA-Seq and small RNA-Seq using nitrogen-starvation stress-treated rice samples, and analyzed the expression profiling of the non-coding RNAs and their target genes. With this study, we investigate the relationship between the expression of nitrogen-starvation stress-specific non-coding RNAs and their effects on genes in *Oryza sativa*.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A2A2A01045528) and the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ008011), Rural Development Administration, Republic of Korea.*Correspondence: Chanseok Shin, cshin@snu.ac.kr

194-C Transcriptional regulation and non-coding RNA: The Steroid Receptor RNA activator

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The steroid receptor RNA activator (SRA RNA) is a unique non-coding RNA which has been shown to stimulate hormone-mediated transcriptional responses. The SRA RNA is a member of the diverse group of factors known as the nuclear receptor co-regulators. The RNA has a complex secondary structure with multiple stem-loop structures which are the landing platform for its partners. Understanding the specific association between the SRA RNA and its regulatory partners will provide critical tools to modulate hormone-mediated transcriptional responses. Increased understanding of these signalling pathways has great potentials for breast and pancreatic hormone-responsive cancer treatment for example. We are studying the specific association of the SRA RNA with two transcriptional regulators: the SRA stem-loop interacting RNA binding protein (SLIRP) and the *SMRT*/*HDAC*-associated repressor *protein* (SHARP). Both proteins are classified as transcriptional repressors. SLIRP is a small RRM-containing protein shown to specifically associate with SRA in the nucleus and to be a partner of the mitochondrial protein LRPPRC. SLIRP is binding to the SRA RNA and is thought to outcompete other transcriptional activators. The SHARP protein belongs to the split end protein family, initially characterized in Drosophila where it was shown to affect homeotic patterning. SHARP recruits the SMRT protein and histone deacetylases to the promoter regions, which in turn blocks the transcriptional machinery by promoting chromatin compaction.

We are characterizing *in vitro* the binding properties of these factors with their RNA binding sequences in the SRA RNA. Furthermore, we have obtained an atomic model of the N-terminal region of SHARP using X-ray crystallography. This region contains three RRM domains (RRM2, RRM3 and RRM4) which are conserved within the entire SHARP protein family. Two of these RRMs (RRM3-4) are forming a stable entity using a previously uncharacterized region. The third RRM (RRM2) is tethered to the others via a helical linker. Small angle X-ray scattering experiments indicates that the linker could be used by the protein single RRM to reach two preferential orientations located on both side of the stable block formed by the RRM3-4. Structural comparison, mutagenesis and *in vivo* experiments are validating the importance of the RRM organization and binding ability for the co-repressor activity mediated by SLIRP and SHARP.

197-C Evolutionary relationships between PB1 mRNA of Influenza A virus and host microRNAs among vertebrates

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Influenza A virus (IAV) undergoes rapid evolution to evade the host defense system, whereas hosts have developed several antiviral responses. Recently, it has been reported that three human miRNAs (miR-323, miR-491 and miR-654) are able to bind to almost the same site in the viral polymerase basic protein 1 (PB1) mRNA and inhibit H1N1 IAV replication (Song *et al*., *J. Virol*. 2010). Thus, these miRNAs have been suggested as potential novel antiviral factors against IAV. Meanwhile, some viruses are known to subvert host antiviral function of miRNAs, indicating that miRNAs play critical roles in complicated host-pathogen interaction networks.

To better understand the intricate host-IAV interactions via miRNAs, here, we investigated the evolutionary relationships between host miRNAs and PB1 mRNA. Firstly, to characterize the dynamic nature of human IAV evolution, we classified a variety of human IAV (7,368 strains) from 1918 to 2012 based on the amino acid sequence similarities. Consequently, large-scale spectral clustering analysis visualized a comprehensive picture of IAV evolution in whole segments, showing not only process of reassortment but also gradual mutations simultaneously. Secondly, conservation analysis of miRNA-target sites in PB1 mRNA sequences based on Shannon's information theory suggested that the miRNA-target sites were significantly conserved in human IAVs from 1918 to 2012. In addition, both swine and avian IAVs (1,412 and 8,257 segments, respectively) also possessed the miRNA-target sequences in PB1 mRNA. As for host miRNAs, both miR-323 and miR-491 were conserved in human and swine genome. Furthermore, we predicted that several other host miRNAs hybridized with the conserved miRNA-target sequences in human, swine and avian.

On the basis of these results, we suggested that regulatory relationships between host and IAV via miRNAs had been evolutionary conserved among vertebrates, and, therefore, we proposed the following hypotheses: (1) host miRNAs targeted immutable regions of IAV, or (2) IAV regulated appropriately its own replication in order to maintain the persistent infection by utilizing the host defense system of miRNAs. In this conference, evolutionary advantage of host miRNA regulations will be discussed from the standpoints of both hosts and viruses.

200-C Noncoding RNA-mediated chromosomal fusions

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Chromosomal fusion, a common occurrence in normal and cancer cells, can lead to the formation of aberrant gene products and chimeric transcripts. The mechanisms driving these fusions are poorly understood, but recurrent fusions are widespread. Here we describe chromosomal fusion events that are driven by either long or short aberrant RNAs during somatic differentiation in the ciliate *Oxytricha trifallax*. Exposure of the germline to either long noncoding "template" RNAs that specify rearrangements (Nowacki et al. 2008 *Nature*) or to 27 nucleotide piRNAs that protect DNA sequences against deletion (Fang et al. 2012 *Cell*) can lead to fusion of chromosomes in the offspring, or even the formation of circular chromosomes, in one case. Furthermore, we demonstrate that these RNA-mediated inter- and intra-chromosomal fusions are heritable over multiple sexual generations, illustrating the ability of noncoding RNAs to program and reprogram genome architecture, including chromosome fusion and circularization, and expanding the known repertoire of RNA-mediated, transgenerational, epigenetic inheritance.

203-C RNA functional profiling by gene deletion in S. cerevisiae

Jian Wu[1], *Steven Parker*[1], *Sara Shamsah*[1], *Daniela Delneri*[1], *Raymond O'Keefe*[1]

[1]**University of Manchester**

New sequencing technologies and high-resolution microarray analysis have revealed that an large portion of the genome is transcribed, generating a significant number of RNAs with non-coding capacity. The focus of current debate is how many of these non-coding RNAs are functional, and what is their function. The yeast *Saccharomyces cerevisiae* is an important model organism for investigating gene functions. Protein-coding gene deletion strains have provided a valuable research resource for studying essential cellular processes and understanding the mechanisms of drug action. However, the pervasively transcribed RNA-coding genes are absent from deletion strain collections, making it difficult to study the contribution of RNA-coding genes in biological processes. Therefore, we have constructed molecular barcoded RNA gene deletion strains in *S. cerevisiae*, including annotated snRNAs, snoRNAs, tRNAs and recently identified stable unannotated transcripts (SUTs) and cryptic unstable transcripts (CUTs). In addition, the verified RNA hemizygotes have been sporulated and dissected to generate haploid mutant collections and homozygous deletion strains. The majority of RNAs studied are non essential under nutrient rich (YPD) conditions. Furthermore, these RNA deletion strains will be used for RNA fitness profiling to explore, by next generation sequencing of barcodes, the role of individual RNAs to growth of yeast under a variety of conditions and treatments. Nevertheless, selected RNA genes will be investigated for their potential roles in regulating expression of genes nearby. Finally, all RNA deletion strains will be deposited with international repositories for yeast strains to allow distribution of this new resource to provide a comprehensive gene deletion collection for genome-wide analysis.

718-C Prostate cancer exosomes offering novel circulating non-coding RNA biomarkers for early cancer diagnosis and prognosis

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Prostate cancer (PCa) can be cured in approx. 80% of men presenting with early, organ confined disease following surgery to remove the prostate. The commonly used blood test for prostate-specific antigen (PSA) shows elevated serum levels in men with PCa. Unfortunately, PSA alone is a poor predictor of disease outcome (prognosis) and invasive prostate biopsies are required to determine the PCa stage and prognosis. As a result, poor testing compliance results in most men presenting with advanced disease which is largely incurable. Thus, we are investigating the utility of RNAs enriched in cellular nanovesicles (40-100nm in size) released into the circulation, to develop novel non-invasive biomarker tests for early PCa diagnosis and prognosis.

Total RNA was isolated from human prostate epithelial cells, established PCa cell lines and their culture supernatant exosomes. The microRNA (miRNA) and messenger RNA (mRNA) expression profiles from these sources were analysed using the Affymetrix and Arraystar microarray platforms and the data were mined using Partek Genomics Suite. Biomarker candidates were then validated using qPCR.

The expression profiles revealed a vast number of differentially expressed miRNAs and mRNAs in PCa exosomes that can be readily developed into potential biomarkers. In brief, many miRNAs were up-regulated in PCa exosomes while the majority of mRNAs were down-regulated. Gene set analyses further suggested that differentially expressed exosomal RNAs were associated with various aspects of cancer development and progression. Exosomes show potential as sources for novel circulating biomarker discovery.

Mechanisms of RNA interference

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 206 C – 221 C
Location: Main Hallway & Sanada Foyer

206 C	**AGO1 requires interaction with GW182 to repress translation of miRNA targets**
209 C	**Identification of anhydrobiosis-related genes using RNA interference with 27-bp RNA duplexes**
212 C	**Analysis on viral suppressors of RNA silencing in plant cell-free RISC assembly system**
215 C	**NMR structural study of the two N-terminal dsRBDs of TRBP in complex with siRNA.**
218 C	**The rough Endoplasmatic Reticulum is the central nucleation site of siRNA-mediated RNA silencing**
221 C	**Computational Analyses and Experiments Indicate Role of Guide RNA Structure in MicroRNA::Target Interaction**

206-C AGO1 requires interaction with GW182 to repress translation of miRNA targets

Andreas Boland[1], Eric Huntzinger[1], Duygu Kuzuoglu-Öztürk[1], Maria Fauser[1], Elisa Izaurralde[1]

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Animal miRNAs silence the expression of mRNA targets through translational repression, deadenylation and subsequent mRNA degradation. Silencing requires association of miRNAs with an Argonaute protein (AGO) and a GW182 family protein. In turn, GW182 proteins interact with PABP and the PAN2-PAN3 and CCR4-NOT deadenylase complexes. These interactions are required for the translational repression, deadenylation and decay of miRNA targets (Huntzinger at al., 2013). Recent studies have indicated that miRNAs can also repress translation in a GW182-independent but AGO-dependent manner (Fukaya and Tomari, 2013). AGO1 and GW182-mediated repression was reported to occur by distinct mechanisms in *Drosophila melanogaster* (Dm) cells (Fukaya and Tomari, 2013). However, the contribution of these two alternative mechanisms to silencing has remained unclear. To address this question, we characterized the interaction of *Dm* AGO1 and GW182. Based on the recent structure of human AGO2 (Schirle and MacRae, 2012), we designed mutations that disrupt *Dm* AGO1 interaction with GW182. Functional assays in *Dm* cells indicate that *Dm* AGO1 requires interaction with GW182 to mediate translational repression and degradation of mRNA targets and does not possess independent repressive activity. We are combining cellular, biochemical and structural approaches to investigate how the GW182 proteins repress translation of miRNA targets.

209-C Identification of anhydrobiosis-related genes using RNA interference with 27-bp RNA duplexes

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[1]University of São Paulo; [2]University of Cambridge

Anhydrobiosis is a highly stable state of biological organization that is achieved by certain species when exposed to extreme water stress. During anhydrobiosis, specimens can be submitted to diverse abiotic stresses such as UV light, radiation and pressure, and still remain viable. RNA interference (RNAi) has been used to identify anhydrobiosis-related genes in order to allow the development of future technologies enabling more efficient methods for conserving organs, vaccines, enzymes and molecules of interest.

RNAi can be triggered in the anhydrobiotic nematode *Panagrolaimus superbus* through many ways, as i) micro-injection, ii) feeding or iii) soaking. We decided to do a screening of 25 genes that have been described as up-regulated during desiccation in other anhydrobiotic species, using soaking with 27 bp siRNAs (dicer substrates), approach that is much simpler since dicer substrates can be commercially purchased and was shown by our group as an efficiently technique of triggers RNAi.

Worms were grown at 20°C in the dark, collected from culture plates and transferred to tubes containing siRNAs against a target gene in the final concentrations of 1 mM. They were kept in the dark for 24 hours at 21°C. Dicer substrates were designed in the program Strand Analysis. After soaking, to determine whether the knockdown leads to lethal effects, survival percentages were determined by staining with Erythrosin B. To determine the effect of RNAi knockdown on desiccation resistance, the worms were immobilized on 0.45 µm Supor filter membranes by vacuum filtration with a Sartorius funnel, then placed into 1.5 mL test tubes. Worms were then subjected to the following conditions: 98% relative humidity (RH) for 24 h (in a saturated solution of copper sulphate); 10% RH for 24 h (in dry silica gel) and pre-hydration in 100% RH for 24 h (in distilled water vapour). Rehydration was achieved by adding 1.5 mL of M9 buffer to the tubes. Determination of viability after desiccation was done by staining with Erythrosin B.

On this screening, we were able to identify that 12% of the selected genes showed significant reduction in viability after silencing and desiccation. This study is important to guide our next steps on the comprehension of the anhydrobiotic process. Our findings may accelerate the process of identifying anhydrobiosis-related genes.

212-C Analysis on viral suppressors of RNA silencing in plant cell-free RISC assembly system

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Small RNAs including small interfering RNAs (siRNAs) and microRNA (miRNAs) play large roles in both plant and animal gene expression. The effector complex of posttranscriptional gene silencing (PTGS), RNA-induced silencing complex (RISC), contains a single-stranded small RNA bound to an ARGONAUTE family protein (AGO). RISC represses gene expression, guided by the small RNA to complementary sequences on target RNAs. RISC assembly is a key step of RNA silencing. On plant RISC assembly, small RNA duplexes are loaded onto AGOs in a manner dependent on molecular chaperone machinery, followed by removal of one strand of the duplex. PTGS is an important mechanism for antiviral defense in plants. To counteract this defense, viruses express viral suppressors of RNA silencing (VSRs). Despite accumulating evidence of VSRs, it is not clear how VSRs act on host PTGS. The VSR functions have not been hitherto addressed in a plant cell-free system that recapitulates RISC assembly. This study analyzes the roles of several VSRs, including *Tomato bushy stunt virus* P19, *Turnip crinkle virus* P38, *Peanut clump virus* P15, *Cucumber mosaic virus* 2b, and *Potato virus Y* HC-Pro, in a cell-free RISC assembly system that has been recently developed by using extracts of evacuolated tobacco BY-2 protoplasts (BYL). All VSRs synthesized by *in vitro* translation inhibited RISC assembly, but did not affect target RNA cleavage by preassembled RISC. The inhibitory effect of VSR on RISC assembly was different among tested synthetic small RNA duplexes. The present study can reveal novel characteristics of VSRs, and further help dissect molecular mechanisms underlying PTGS.

215-C NMR structural study of the two N-terminal dsRBDs of TRBP in complex with siRNA.

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RNA interference mediated by small interfering (si)- or micro (mi)- RNA is a cellular mechanism of gene expression regulation at the post-transcriptional level. The molecular effector is the RISC (RNA Induced Silencing Complex), a ribonucleoparticle comprising the protein Argonaute (Ago) and a single strand RNA called the siRNA 'guide strand'. Messenger-RNA recognition by the RISC, occurring through the sequence specific annealing with the guide strand, leads to translation inhibition and/or mRNA degradation.

Si-RNA guide strand precursors are RNA duplexes of ca. 20 base pairs. However, only one strand is selected and loaded into Ago during each RISC formation event. For a particular class of siRNA called asymmetric siRNA, the guide strand selection process is strongly biased, with one strand of the siRNA duplex being preferentially loaded into Ago. Although this property has been shown to correlate with the thermodynamics stability of the siRNA extremities, the molecular mechanism of the selection process is not understood yet. The key protein factors involved in the guide strand selection process are Ago, TRBP, and Dicer, associated into the RLC (RISC Loading Complex), but the relative contribution of these is yet unknown.

In order to understand better the contribution of TRBP in RISC loading, we carried out a structural study of its two N-terminal dsRBDs (dsRNA Binding Domains) (dsRBD12) in complex with various siRNAs by NMR spectroscopy. Three-dimensional structures of the each dsRBD were solved in their RNA bound form, revealing the presence of an additional a-helix at the N-terminus of the first dsRBD. Intermolecular contacts observed between dsRBD12 and a highly asymmetric siRNA indicate the presence of two major dsRBD12 binding sites. Furthermore, RDC (Residual Dipolar Coupling) data measured in two different media suggest that the two dsRBDs of TRBP do not bind siRNA independently, but rather adopt a well-defined orientation relative to one another. Interested by the impact of siRNA methylation in silencing activity, we tested whether siRNA methylation would also have an impact on TRBP-siRNA binding. Our NMR data show that position specific methylation of RNA actually affects TRBP binding on siRNA. Overall, by helping to characterize siRNA recognition by TRBP, our results contribute to the understanding of the role of TRBP in RNA silencing.

218-C The rough Endoplasmatic Reticulum is the central nucleation site of siRNA-mediated RNA silencing

Lukas Stalder[1], Wolf Heusermann[6], Lena Sokol[5], Dominic Trojer[4], Anja Fritzsche[4], Jan Weiler[4], Martin Hintersteiner[2], Florian Aeschimann[3], David Morrissey[4], Nicole Meisner Kober[4]

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Despite of progress in mechanistic understanding of the RNA interference (RNAi) pathways, the subcellular sites of RNA silencing still remain under debate. We will present a quantitative tracing of the fate of exogenous siRNA within the cell upon lipid delivery. Our data demonstrate that entry of siRNA in the RNAi pathway with current delivery vehicles is highly inefficient, whereas trafficking of the siRNA to the site of RISC (RNA induced silencing complexes) loading emerges as one major rate limiting step. We will further present data which reveal that loading of lipid-transfected siRNAs and endogenous microRNAs into RISC, encounter of the target mRNA, and Ago2-mediated mRNA slicing in mammalian cells are nucleated at the cytosolic membrane of the rough endoplasmic reticulum (rER). We identify TRBP and PACT as key factors anchoring RISC to ER membranes in an RNA-independent manner. Finally we will discuss implications of our work on the development of next generation, subcellular targeted delivery strategies for RNAi therapeutics.

221-C Computational Analyses and Experiments Indicate Role of Guide RNA Structure in MicroRNA::Target Interaction

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MicroRNAs (miRNAs) are small noncoding RNAs which regulate expression of numerous metazoan genes posttranscriptionally. Binding of mature miRNAs guides RNA-induced silencing complexes to complementary messenger RNA targets. We investigated the role of guide structures in miRNA::target interactions using computational RNA secondary structure analyses and experimental gene knockdown assays. The computational investigation revealed characteristic common and species-specific features. Virtually all structures contained at least one unpaired site suitable for nucleation suggesting that the kinetics of RNA-RNA interactions contribute to RISC binding. Structures of mature human miRNAs were found to have a preference for U/A-rich unpaired 5' ends and U/G-rich paired 3' ends. Unpaired sites matching with validated targets were rich in base-pairing-competent bases G and U and poor in A and C as compared to nonmatching sites indicating the involvement of such sites in miRNA::target interactions. One group of miRNAs preferred a single nucleation site, located either 5' terminal, 3' terminal or central, whilst others appeared to be bi- or multivalent. Conversely, local mRNA targets allowed only one kind of nucleation indicating that mRNAs select their miRNA partners as much as the other way around. We experimentally confirmed this hypothesis by comparing wild-type miRNAs with mutants in which target base-pairing was unchanged but which comprised reprogrammed guide structures and nucleation sites. Removal of nucleation sites resulted in reduced silencing, whereas switch from one to another allowed interactions, did not affect silencing. Nucleation site changes yielding site extensions enhanced silencing. We suggest a two-phase control mechanism of miRNA::target interactions: First, a kinetic nucleation-based control dictated by the mRNA target site repelling false-positive seed matches and second a thermodynamically controlled seed match verification process dominated by the sequence of the mature miRNA.

RNA and Epigenetics

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 224 C – 230 C
Location: Main Hallway & Sanada Foyer

224 C **Structural Characterization of the RNA, Cyp33, MLL, Histone H3 Interaction Network**
227 C **Histone replacement reveals distinct essential functions for H3K36 methylation**
230 C **Regulation of piRNA production**

224-C Structural Characterization of the RNA, Cyp33, MLL, Histone H3 Interaction Network

Markus Blatter[1], *Charlotte Meylan*[1], *Frédéric H.-T. Allain*[1]

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The Cyclophilin 33 (Cyp33) is a member of the *cis-trans* peptidyl-prolyl isomerases family of cyclophilins. Members of this family possess various additional domains. Cyp33 includes an N-terminal RNA Recognition Motif (RRM) domain next to the conserved cyclophilin domain with PPIase activity.

It was shown that the RRM domain of Cyp33 mutually exclusive binds to RNA and the third Plant Homeobox domain (PHD3) of the Mixed Lineage Leukemia factor (MLL) [1]. This competitive binding makes Cyp33 unique among the RNA recognition motif. Further it was demonstrated that the binding of Cyp33 to the Mixed Lineage Leukemia factor down-regulates the expression of some targets of MLL, which is a transcription activator for a series of genes from the HOX family. RNA binding sequences of Cyp33-RRM can be found in an non-coding RNA (ncRNA) located in the 3'-UTR of HOXC8, which itself is a target of MLL. Addition of this ncRNA rescues the expression of HOXC8 in cells with a silent HOXC locus leading to the theory that Cyp33 bound to MLL is sequestered by the ncRNA and thereby allow MLL to switch back to it activation state. In its transcriptional active state MLL is bound to a specific epigenetic mark on Histone H3 (H3K4me3) mediated by the PHD3 domain, the same domain which also interacts with the RRM of Cyp33. MLL is an oncoprotein and it fusion variants are associated with infant leukemia. All of the fusion variants of MLL lack the cassette of the homeobox and all subsequent domains. It was shown that reinsertion of the third PHD domain (PHD3) rescues the aberrant transcription caused by MLL fusion proteins [2]. This emphasizes the importance of this interaction network between RNA, Cyp33, MLL and H3K4me3.

In this project we studied the various structural aspects of all four component of the beforehand described interaction network using Nuclear Magnetic Resonance spectroscopy and Isothermal Titration Calorimetry. Using different labeling schemes we expressed the RRM domain of human Cyp33 and in addition to the PHD3 domain of human MLL. Further we used different RNAs (AAUAAA and UAAUGUCG) and the Histone H3 peptide with ammonium tri-methylation at lysine four (H3K4me3).

The structure of Cyp33-RRM in free state revealed a third alpha helix in the conserved C-terminal extension of the RRM which folds back on the RRM beta-sheet. Different binding registers precluded an accurate structure determination of the RRM bound to RNA. After all we obtained a model for such a complex which shows that the third helix has to dissociate from the beta-sheet in order to allow complexation with RNA. Structure determination of the Cyp33-RRM : MLL-PHD3 complex in combination with ITC experiments involving H3K4me3 revealed an allosteric effect in ternary complex formation of Cyp3-RRM, MLL-PHD3 and the epigenetically marked tail of Histone H3.

1. Wang, Z., et al., Cell, 2010. **141**(7): p. 1183-94.

2. Muntean, A.G., et al., Blood, 2008. **112**(12): p. 4690-3.

227-C Histone replacement reveals distinct essential functions for H3K36 methylation

Michael Meers[1], *A. Gregory Matera*[2]

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Histone post-translational modifications (PTMs) are known to function importantly in regulating gene expression, primarily at the level of DNA promoters and cis-regulatory elements. However, recent evidence suggests that PTMs may also play important co-transcriptional roles related to RNA processing. Methylation of lysine 36 of the histone H3 subunit (H3K36) in particular has been implicated in co-transcriptional suppression of cryptic transcription initiation and, more recently, in regulation of alternative splicing. Though the complexity of co-transcriptional RNA-level regulation necessitates a robust, controlled *in vivo* model to study the effect of depleting histone PTMs upon splicing, to date such a model has not been developed. Crucially, existing studies that link H3K36 methylation with RNA processing rely upon perturbation of PTM-catalyzing "writer" enzymes, many of which are known to have non-histone substrates that can confound interpretation of phenotypes. Here we present a novel set of tools in *Drosophila melanogaster* that enable full genetic replacement of endogenous histones with transgenic copies bearing PTM-inactivating mutations. We have generated endogenous histone deletion lines complemented by transgenic histones containing mutant H3K36 alleles to facilitate *in vivo* analysis of the RNA processing consequences of eliminating H3K36 methylation. We report that two H3K36 alleles, alanine (H3K36A) and arginine (H3K36R), rescue full histone deletion phenotypes to vastly different degrees, suggesting a separation of essential functions for H3K36. Interestingly, H3K36R animals exhibit locomotion defects two days prior to lethality, suggesting potential defects in neuronal processes in which alternative splicing is known to play a prominent role. We intend to use RNA-seq to comprehensively evaluate expression and splicing phenotypes manifested in H3K36 alleles.

230-C Regulation of piRNA production

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Setting germ cells apart from other somatic cells in the body is crucial for all sexually reproducing organisms. This process of germ cell specification occurs early in embryogenesis and requires the conserved process of transcriptional silencing. In *Drosophila* germ cells global transcriptional silencing is mediated by the gene *polar granule component* (*pgc*) that codes for a small 7 kDa protein. *pgc* mutant germ cells show precocious active chromatin marks that result in the transcription of somatic genes that lead to their death. Thus transcriptional silencing mediated by *pgc* feeds into the epigenetic pathway and plays a critical role in specifying a germ cell fate by suppressing a somatic one. We have found that in *Drosophila* repressive transcriptional mechanisms also play an important role at a different stage of germ cell development, namely germ line stem cell (GSC) differentiation. Repressive marks consistent with heterochromatin, namely H3K9me3, H4K20me3 and HP1, are enriched in the nuclei of the differentiating GSC daughter. Interestingly, H3K9me3 enriched chromosomal sites correlate with the location of piRNA production. The piRNA pathway is a small RNA-based mechanism that is a major component of the defense against transposable elements (TE) in the germ line. In mutants defective in heterochromatin formation, differentiation of GSC is blocked, piRNA levels are reduced, and TE activity is increased. While we thus know that heterochromatin protects the germ line by activating the piRNA pathway and repressing transposable elements, we do not understand what controls heterochromatin formation in the GSC daughter. Surprisingly, we have observed that the transcriptional silencer *pgc* that has a known role in germ cell specification is also expressed during GSC differentiation. We hypothesize that *pgc* causes transient transcriptional silencing that allows heterochromatin formation on the piRNA producing clusters and thus permits proper GSC differentiation. We have shown that the expression of the transcriptional silencer *pgc* in the GSC daughter is controlled by translational control factors Nanos and Pumilio.

Small RNAs

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 233 C – 257 C
Location: Main Hallway & Sanada Foyer

233 C	**Common regulation of micro-RNAs by oncogenic transcription factors in B-cell lymphomas**
236 C	**Quantitative analysis reveals extensive target specificity of individual let-7 miRNA family members in vivo**
239 C	**Characterization of microRNAs derived from the HIV-1 TAR RNA hairpin**
242 C	**Highly potent and specific siRNAs isolated from E. coli with endogenous p19 expression**
245 C	**X-ray crystal structure of Maelstrom**
248 C	**Molecular mechanisms of the piRNA biogenesis machinery**
251 C	**Sensitized Backgrounds Reveal Critical Roles for microRNA Families**
254 C	**Novel stress-induced smRNAs from Brachypodium distachyon**
257 C	**Nonstop decay in C. elegans: examination of a possible role for 22G RNAs**

233-C Common regulation of micro-RNAs by oncogenic transcription factors in B-cell lymphomas

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micro-RNAs (miRNA) are regulators of many processes including the development and maintenance of cancer. Many important targets of oncomiRs and tumor suppressive miRNAs have been identified; however, the regulation of these miRNAs in cancer is not well understood. Here we demonstrate the common regulation of miRNAs by the oncogenes v-Rel, c-Myc and the AP-1 transcription factor family. We performed miRNA-seq analysis, comparing v-Rel and c-Myc transformed chicken B-cell lines to a normal bursal (B-cell) control and have identified many miRNAs that are differentially regulated. More miRNAs were upregulated than downregulated in this analysis. Using EST databases and ChIP-seq data generated by the ENCODE project, we predicted the promoters of 600 human miRNAs and found that they are conserved across vertebrates. Further, Rel, Myc, Jun and Fos bind promoters of a common set of human miRNAs. Interestingly, we found that most miRNAs upregulated by v-Rel and c-Myc had binding sites in human ChiP-seq data, indicating conserved regulation of miRNAs in chickens and humans. Furthermore, some of the downregulated miRNAs inhibit Rel transformation capability in an in vitro transformation assay.

236-C Quantitative analysis reveals extensive target specificity of individual let-7 miRNA family members in vivo

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Recognition and silencing of miRNA targets is thought to rely chiefly on basepairing of the miRNA 'seed' (nucleotides 2...8) with target sequence. Since miRNAs can occur in 'families', defined by a shared seed sequence, members of a given family are considered to function interchangeably in silencing specific targets. Here, we have used the *let-7* miRNA family in *C. elegans* as a paradigm to test this hypothesis rigorously and under physiological conditions. To this end, we have developed a quantitative imaging assay that examines the effects of endogenous miRNAs on reporter transgenes, which we express in various tissues at defined, constitutive but low (physiological) levels through targeted single copy transgene integration. We find that *let-7* and its sisters act in a strikingly non-redundant manner. In particular, when examining a number of distinct targets, we observe that they differ not only in their spatial repression patterns but also in their response to the loss of individual *let-7* family members. This specificity is a function of target site sequence, which we demonstrate by reengineering a target of *let-7 proper* to be repressible by distinct family members. Importantly, family member specificity is extensive but not absolute and appears to further depend on the cellular levels of a miRNA. Thus, miRNA targeting may simultaneously be more restricted as well as dynamic (e.g. across tissues, developmental stages, or in response to environmental cues) than anticipated, impacting on both our understanding of miRNA regulatory networks and experimental procedures for reliable and meaningful miRNA target validation.

239-C Characterization of microRNAs derived from the HIV-1 TAR RNA hairpin

Alex Harwig[1], *Ben Berkhout*[1], *Atze Das*[1]

[1]Lab. of Experimental Virology

The transacting responsive (TAR) hairpin is present at the 5' and the 3' end of the HIV-1 RNA genome. This TAR hairpin is essential for HIV-1 replication because it binds the viral Tat transcriptional activator protein. Several groups have demonstrated the presence of small TAR-derived RNAs in HIV-1 infected cells. Supposedly, these small RNAs are microRNAs (miRNAs) produced by the RNAi pathway. We used the sensitive SOLiD ultra-deep sequencing method to characterize these small RNAs in HIV-1 expressing cells. This analysis revealed the presence of miRNAs corresponding to both the 5' and the 3' side of the TAR stem, with the 3' fragments being more abundant. The cleavage pattern of the miRNAs differed from the patterns expected for the Drosha/Dicer mediated RNAi pathway and suggests the involvement of another nuclease. Northern blot analysis of the intracellular RNA confirmed the presence of the TAR miRNAs and their TAR RNA precursor. Analysis of TAR-mutated HIV-1 variants revealed that these TAR RNAs and miRNAs are produced exclusively from the TAR element present at the 5' end of the viral transcripts. The role of Tat in the production of these small RNAs is currently investigated.

242-C Highly potent and specific siRNAs isolated from E. coli with endogenous p19 expression

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Most synthetic siRNAs have been produced by chemical synthesis. Here we present a method to produce highly potent siRNAs in E.coli. This method relies on ectopic expression of p19, an siRNA-binding protein found in a plant RNA virus. When expressed in E. coli, p19 stabilizes an ~21-nt siRNA-like species produced by bacterial RNase III. These siRNAs are then isolated from bacterial total RNA by binding to p19-coupled magnetic beads. When mammalian cells are transfected by siRNAs generated in bacteria expressing p19 and a hairpin RNA encoding 200 or more nucleotides of a target gene, they selectively knock down expression of the specific target gene by ~90% without immunogenicity or off-target effects. The bacterial expression of p19 can also be used to isolated novel endogenous siRNAs made from anti-sense transcripts.

245-C X-ray crystal structure of Maelstrom

Naoki Matsumoto[1], *Hiroshi Nishimasu*[1], *Kaoru Sato*[1], *Ryuichiro Ishitani*[1], *Haruhiko Siomi*[2], *Mikiko Siomi*[1], *Osamu Nureki*[1]

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Transposons occupy a large part of our genome and the mobilization of these elements induces genetic instability. PIWI-interacting RNAs (piRNAs) associate with PIWI proteins and silence transposable elements, thus maintaining the genomic integrity of the germ line. Maelstrom (Mael) plays a critical role in the piRNA pathway. Mael consists of a HMG-box domain and a MAEL domain. The HMG-box domain binds the *miR-7* promoter and represses *miR-7* expression, ensuring proper differentiation of *Drosophila* germline stem cell lineage. Bioinformatics analysis suggested that the MAEL domain has an RNase H-like fold but lacks catalytic residues highly conserved among RNase H proteins. The MAEL domain is also characterized by a conserved ECHC motif. A recent study using ovarian somatic cells (OSCs) showed that Mael participates in Piwi-mediated epigenetic regulation of transposons but is not required for piRNA biogenesis. However, the mechanism by which Mael is involved in the piRNA pathway remains unknown. Here, we report the crystal structure of the *Drosophila melanogaster* MAEL domain at 1.6 A resolution. The crystal structure revealed that the MAEL domain has an RNase H-like fold with a zinc ion coordinated by the ECHC motif. A structural comparison of the MAEL domain with RNase H proteins showed that the MAEL domain lacks any catalytic residue. Instead, the MAEL domain has a conserved molecular surface, which may serve as a platform for interacting with nucleic acids and/or proteins in the nucleus of OSCs. Functional analyses based on the crystal structure are undergoing.

248-C Molecular mechanisms of the piRNA biogenesis machinery

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Epigenetic silencing of transposons by Piwi-interacting RNAs (piRNAs) constitutes an RNA-based genome defense mechanism. Piwi endonuclease action amplifies the piRNA pool by generating new piRNAs from target transcripts by a poorly understood mechanism. Here, we identified mouse Fkbp6 as a factor in this biogenesis pathway delivering piRNAs to the Piwi protein Miwi2. Mice lacking *Fkbp6* derepress LINE1 (L1) retrotransposon and display reduced DNA methylation due to deficient nuclear accumulation of Miwi2. Like other co-chaperones, Fkbp6 associates with the molecular chaperone Hsp90 via its tetratricopeptide repeat (TPR) domain. Inhibition of the ATP-dependent Hsp90 activity in an insect cell culture model results in the accumulation of short antisense RNAs in Piwi complexes. We identify these to be by-products of piRNA amplification that accumulate only in nuage-localized Piwi proteins. We propose that the chaperone machinery normally ejects these inhibitory RNAs, allowing turnover of Piwi complexes for their continued participation in piRNA amplification.

251-C Sensitized Backgrounds Reveal Critical Roles for microRNA Families

Elizabeth Jeanne Thatcher[1], *Victor Ambros*[2]

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MicroRNAs (miRNAs) are a class of small regulatory RNAs that have been implicated in the control of many cellular functions including cell specification, differentiation, proliferation, and metabolism. A smaller number of miRNAs are expressed in stem cells and at early stages of development, including some that are thought to maintain pluripotency. Interestingly, even though many *C. elegans* miRNAs are highly conserved, for many single deletion knockouts or even family knockouts, phenotypes are subtle or not evident. *C. elegans* have 23 miRNA families, 9 of which are conserved through humans. The Horvitz group showed that most miRNAs are not essential for development or viability on their own. This indicates a high degree of functional redundancy among miRNA family members, and between unrelated miRNA families in the worm. Recent findings from the Abbott group illustrated that sensitizing the worm may reveal several mutant phenotypes associated with loss of individual miRNAs or families.

Here, I examined the phenotypes associated with several critical miRNA effector complexes to determine whether combining reduced effector complex expression with miRNA family knockouts were able to alleviate or exacerbate the observed phenotypes. For instance, RNAi of Alg-1 or Alg-2 (Argonaute-like genes) produces characteristic developmental delays and disruptions that include problems with molting, slow growth, absent or missing alae, and protruding vulva. These abnormalities are occasionally severe enough to produce embryonic or larval lethality. Examining the effects of Alg-1 or Alg-2 RNAi on miRNA family mutants produces an interesting picture. Most notably, the loss of some miRNAs appears to alleviate the impact of RNAi on Alg-1 or Alg-2 to produce a less severe phenotype as compared to RNAi on wild-type animals. Moreover, some family mutants are uniquely sensitive to Alg-1 versus Alg-2 RNAi or vise versa. These results produce a larger picture of the interaction of unique miRNA families with different effector molecules that allow for a tightly regulated environment to control highly specific and reproducible events throughout development.

254-C Novel stress-induced smRNAs from Brachypodium distachyon

Hsiao-Lin V. Wang[1], *Brandon L. Dinwiddie*[1], *Herman Lee*[1], *Julia Chekanova*[1]

[1]University of Missouri-Kansas City

Small non-coding RNAs (smRNAs), which include miRNAs, siRNAs, as well as other classes of endogenous smRNAs, have been firmly established as key regulators of the cellular and genome functions in diverse eukaryotic organisms, including all land plants. However, it is challenging to study common monocot plants in standard laboratory settings due to their large genomes, physical size, as well as demanding growth conditions. During the past decade, *Brachypodium distachyon* has emerged as a new model organism for studying functional genomics of grasses. The goal of this study was to investigate how smRNA transcriptome of temperate grasses responds to various biotic and abiotic stresses.

To address this question *Brachypodium* plants were challenged with different abiotic stresses such as heat, cold and salt, as well as with infection by the plant pathogen fungus *Magnaporthe grisea*, and their smRNA transcriptomes were examined by deep sequencing. To analyze the populations of smRNAs we deployed proximity-based algorithm, and also classified smRNAs on the basis of their size, the nature of their first nucleotide, and their genomic features. We found that challenging plants with stresses resulted in upregulation of the population of smRNAs ranging from 21 to 25nt in length, while the populations of smRNAs shorter than 20nt in length were downregulated.

In addition to previously reported plant miRNAs and siRNAs, we also identified new groups of stress-specific smRNAs. Among the group of upregulated smRNAs we discovered a class of novel 24nt smRNAs. These smRNAs exhibit several distinct patterns and originate in a strand specific manner from 3'-UTRs of a subset of mRNA genes. The analysis of this group of stress-induced smRNAs and their targets will be presented.

257-C Nonstop decay in C. elegans: examination of a possible role for 22G RNAs

Elaine Youngman[1], *WeifengGu* [2], *Craig Mello*[3]

[1]Villanova University; [2]UMass Medical School; [3]UMass Medical School and Howard Hughes Medical Institute

In *C. eleg*ans, small noncoding RNAs known as 22G RNAs are synthesized by RNA-dependent RNA polymerases and target thousands of protein-coding mRNAs, repetitive sequences, and cryptic transcripts throughout the genome. In some cases, 22G RNA synthesis appears to be triggered as a secondary event downstream of targeting by *C. elegans* piRNAs (known as 21U RNAs) in a pathway that defends against non-self RNA species. (*1, 2*) However, the synthesis of the majority of 22G RNAs is unaffected by loss of 21U RNAs, and the triggers for biosynthesis of these 21U-independent 22Gs remain elusive. Using a comparative genomic approach in geographically distributed wild isolates of *C. elegans*, we have identified a locus (F43E2.6) at which loss of the stop codon is correlated with increased production of 22G RNAs. The F43 mRNA has a short 3'UTR with no further in frame stop codons, raising the intriguing possibility that 22G RNAs could be involved in a nonstop decay pathway in *C. elegans*. F43 is also targeted by an unusual Dicer product; however this Dicer product does not appear to act as a primary siRNA to trigger F43 22G RNAs. First, the Dicer product is expressed equally in strains with large differences (more than 10 fold) in 22G RNA levels. Second, this product is expressed only during embryogenesis, whereas F43 22G RNAs are expressed most strongly in adults. We have demonstrated that the 22G RNA expression phenotype at the F43 locus is genetically linked to the locus itself, and are currently assaying transgenic worms carrying F43 reporter constructs that bear or lack a stop codon to determine whether the loss of a stop codon is indeed causal for increased 22G RNA production. In addition, we are generating reporter constructs that will allow us to ask whether 22G RNAs play a functional role in the nonsense-mediated and no-go decay pathways.

RNA Catalysis and Riboswitches

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 260 C – 269 C
Location: Main Hallway & Sanada Foyer

260 C	**Characterization of Metal Ion Binding Sites in the P4 Helix of Bacillus subtilis RNase P**
263 C	**In vitro evolution of a calcium ion-sensing ribozyme from the natural glmS riboswitch-ribozyme**
266 C	**Tuning the btuB riboswitch fold by chemically modifying its ligand coenzyme B12**
269 C	**Formation of a catalytic supramolecular RNA 1D-array through self-assembly of an engineered group I intron RNA enzyme**

260-C Characterization of Metal Ion Binding Sites in the P4 Helix of Bacillus subtilis RNase P

Yu Chen[1], Xin Liu[1], Carol A. Fierke[2]

[1]Department of Chemistry, University of Michigan; [2]Department of Chemistry and Department of Biological Chemistry, University of Michgan

The endoribonuclease P, RNase P, is responsible for catalyzing the 5'-end maturation of precursor tRNAs. Like many large ribozymes, divalent ions stabilize the folded structure and enhance catalytic function of RNase P. P4 helix, the most highly conserved region in PRNA, is essential for RNase P activity and has been suggested to contain catalytic and/or cocatalytic metal ion binding sites. The crystal structure of *T. maritima* RNase P (4.2 Å) proposed a metal ion directly coordinates the O4 of the bulged uracil (U51 in *B. subtilis*) in the P4 helix[1]. NMR spectroscopy of a P4 stem-loop mimic suggested an inner-sphere metal interaction with the O6 of the base corresponding to G379 in the P4 helix of *B. subtilis* RNase P[2].

To evaluate the structure and function of these putative metal ion sites, 4-thiouracil and 2-aminopurine are specifically substituted for U51 and G379, respectively. These substitutions decrease the pre-tRNA binding affinity at low concentration of metal ion but not at high concentration. Single turnover kinetics show that these substitutions do not affect the rate of the conformational change step in the RNase P - pre-tRNA complex. However, the 4-thiouracil substitution at U51 and 2-aminopurine substitution at G379 decrease the cleavage rate constant in Mg^{2+} by 20-fold and 10-fold, respectively, without altering the $K_{1/2}$ for the Mg^{2+}-dependent activation of cleavage. These data indicate that O4 of U51 and O6 of G379 are important both for stabilizing pre-tRNA affinity in a metal-dependent fashion and for enhancing catalytic activity. The magnitudes of these effects are smaller than expected for an inner-sphere metal ion interaction but are consistent with outer-sphere metal ion coordination.

In contrast, the rSpacer substitution of U51 does not affect the cleavage step but decreases the conformational change rate by 6-fold. Furthermore, the rSpacer substitution of G379 decreases the cleavage rate constant by 3-fold. The modest effect of deletion of the base of U51 and G379 might be due to additional water molecules filling the space allowing a catalytic metal ion to be optimally positioned. These results further suggest that O4 of U51 and O6 of G379 form hydrogen bonds with waters coordinated to a magnesium ion.

1. Reiter, N. J., Osterman, A., Torres-Larios, A., Swinger, K. K., Pan, T. & Mondragón, A. (2010). Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA. Nature 468, 784-789.

2. Koutmou, K. S., Casiano-Negroni, A., Getz, M. M., Pazicni, S., Andrews, A. J., Penner-Hahn, J. E., Al-Hashimi, H. M. & Fierke, C. A. (2010). NMR and XAS reveal an inner-sphere metal binding site in the P4 helix of the metallo-ribozyme ribonuclease P. Proceedings of the National Academy of Sciences 107, 2479-2484.

263-C In vitro evolution of a calcium ion-sensing ribozyme from the natural glmS riboswitch-ribozyme

Matthew Lau[1], *Adrian Ferré-D'Amaré*[1]

[1]National Heart, Lung and Blood Institute, NIH, Bethesda, Maryland 20892-8012, USA

The *glmS* ribozyme is a natural catalytic RNA widely distributed in Gram-positive bacteria that undergoes self-cleavage under physiologic divalent metal ion concentrations upon binding to its cognate coenzyme glucosamine-6-phosphate (GlcN6P) (*1*). This allows bacteria to sense their intracellular GlcN6P concentration and control its biosynthesis at the mRNA level through negative feedback. To address the evolution of its coenzyme dependence, we previously evolved a mutant ribozyme (*glmS*^{AAA}) that is only 3 adenine mutations away from the wildtype ribozyme, and catalyzes the same RNA cleavage chemistry in the absence of GlcN6P. This mutant, in contrast to wildtype, has a strong preference for Ca^{2+}, and requires higher divalent metal ion concentrations (>100 mM) for maximal activity. To further characterize the metal ion specificity of *glmS*^{AAA} and to generate specific metal ion-sensing ribozymes that could function *in vivo*, we have now performed two additional parallel selections. These experiments aimed to evolve self-cleaving ribozymes from *glmS*^{AAA} that are functional in either 2 mM Mg^{2+} or 2 mM Ca^{2+}. From the Mg^{2+} selection, we isolated a fast ribozyme that is equally active in either 2 mM Mg^{2+} or 2 mM Ca^{2+}. The Ca^{2+} selection, on the other hand, produced a Ca^{2+} specific ribozyme that discriminates strongly against Mg^{2+} (more than 10,000-faster in 2 mM Ca^{2+} than in 2 mM Mg^{2+}), and has a higher apparent affinity to Ca^{2+} than *glmS*^{AAA} ($K_{1/2}$ of 19 mM and 93 mM, respectively). This ribozyme is non-specific towards the sequence upstream of its cleavage site, and is not further activated by GlcN6P or physiological concentrations of Mg^{2+}. Our work suggests possible evolutionary links between wildtype *glmS* ribozyme and different ligand independent self-cleaving ribozymes, providing clues as to the origin of the wildtype. Our new selection experiment has generated the first example, to our knowledge, of a self-cleaving ribozyme with such high specificity for Ca^{2+}. We envision that this ribozyme can be engineered to function as an *in vivo* calcium sensor.

This research was supported by the Intramural Research Program of the NIH, NHLBI.

1. W. C. Winkler, A. Nahvi, A. Roth, J. A. Collins, R. R. Breaker, *Nature* **428**, 281 (2004).

266-C Tuning the btuB riboswitch fold by chemically modifying its ligand coenzyme B12

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The focus of our research lies in the binding mechanism between the *btuB* riboswitch of *E. coli* and its ligand coenzyme B_{12} (AdoCbl). [1,2] One interesting fact about this interaction is that the *btuB* riboswitch is highly selective between some derivatives of the B_{12}-family, which however can strongly differ in their general structure.[2,3] In this regard, one of our early findings was that Adenosyl Factor A, which differs from AdoCbl in the shape and position of the lower group, binds and switches the riboswitch with nearly the same affinity and efficiency as the natural substrate. This surprising result lead to the question which are the structural requirements on the large and complex B_{12}-molecule to bind and switch the *btuB* riboswitch.

We have therefore synthesized three series of B_{12}-derivatives modified at the different functional sites (Figure 1). Modifications on the corrin ring sidechains include specific monoacids as well as secondary and tertiary amides. Modifications on the upper ligand include the two natural occurring CN- and Adenosyl-groups as well as synthetic moieties based on platinum(II)-linked nucleobases. A third group comprises derivatives with a peptide backbone replacing the sugar phospate at the lower loop. All these modifications were introduced to study the role of the H-bonding and electrostatic pattern of the *btuB*-B_{12} interaction.

To study the influence of these chemical modifications on the structural rearrangement of the riboswitch we applied in-line probing assays. Our experiments confirmed the importance of an upper moiety, preferentially being adenosyl, for a high affinity to the RNA. The presence of a secondary or tertiary amide group in the sidechains doesn't influence the K_D value but leads to some differences in the structural switch of the RNA. Finally, the derivatives with the lower peptide loop show a strong dependence of the stereochemistry. In fact, only one isomer of this series was shown to switch the riboswitch, although with alterations and with a K_D value in the micromolar range.

Acknowledgments. Financial support by the European Research Council (ERC starting grant 2010 259092-MIRNA to R.K.O.S.) and the University of Zurich is gratefully acknowledged.

[1] W. Winkler, A. Nahvi, R. R. Breaker, *Nature* **2002**, *419*, 952.
[2] S. Gallo, M. Oberhuber, R. K. O. Sigel, B. Kräutler, *ChemBioChem* **2008**, *9*, 1408.
[3] S. Gallo, S. Mundwiler, R. Alberto, R. K. O. Sigel, *Chem. Commun.* **2011**, *47*, 403.

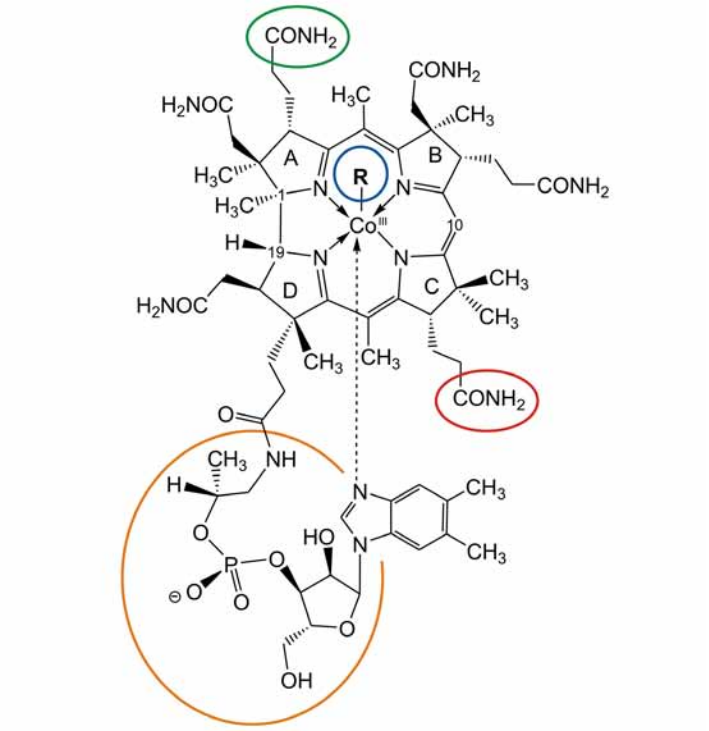


Figure 1: Shematic view of B_{12} with the moieties modified for this study indicated in color. Blue: upper ligand, orange: lower loop, green: sidecain b, red: sidechain e.

269-C Formation of a catalytic supramolecular RNA 1D-array through self-assembly of an engineered group I intron RNA enzyme

Narumi Uehara[1], Hiroyuki Furuta[1], Yoshiya Ikawa[1]

[1]Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University

Self-assembly of biopolymers plays fundamental roles in modern biological systems. DNA duplex structure is essential for storage and replication of genetic information and protein quaternary structure conducts elaborate functions (such as enzyme allosterism) and large complexes (such as virus capsids) of proteins. Self-assembly of polypeptides and nucleic acids are also attractive in the field of nanobiotechnology, in which DNA is regarded as one of the most promising materials for designing 1D, 2D, and 3D nano-objects with defined sizes and shapes.

RNA has been considered as a candidate for nanobiotechnology material because of not only its similarity with DNA but also its functional roles (such as catalysts and receptors) in living cells. Artificial RNA design has produced polygonal and polyhedral RNA nano-objects composed of relatively short (ca. 25-100 nts) RNA oligonucleotides as monomer strands. On the other hand, biological evolution has generated complex RNA 3D structures with sophisticated functions, an extreme example of which is ribosome.

Amalgamation of artificial geometrical RNA nanostructures and naturally occurring RNA 3D structures would generate a new class of RNA nano-objects with enzyme-like functions. In this presentation, we wish to present our recent study to construct self-assembling 1D-nanoarray whose monomer unit is a group I intron RNA. Modular engineering of the *Tetrahymena* group I intron (ca. 400 nts) allowed us to convert its intramolecular interactions for the unimolecular self-folding into the intermolecular interactions for the multimolecular self-assembly, enabling the engineered intron to organize a head-to-tail 1D-assembly. The engineered intron exhibited the catalytic ability depending on the formation of the RNA 1D-array.

RNA structure and folding

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 272 C – 296 C
Location: Main Hallway & Sanada Foyer

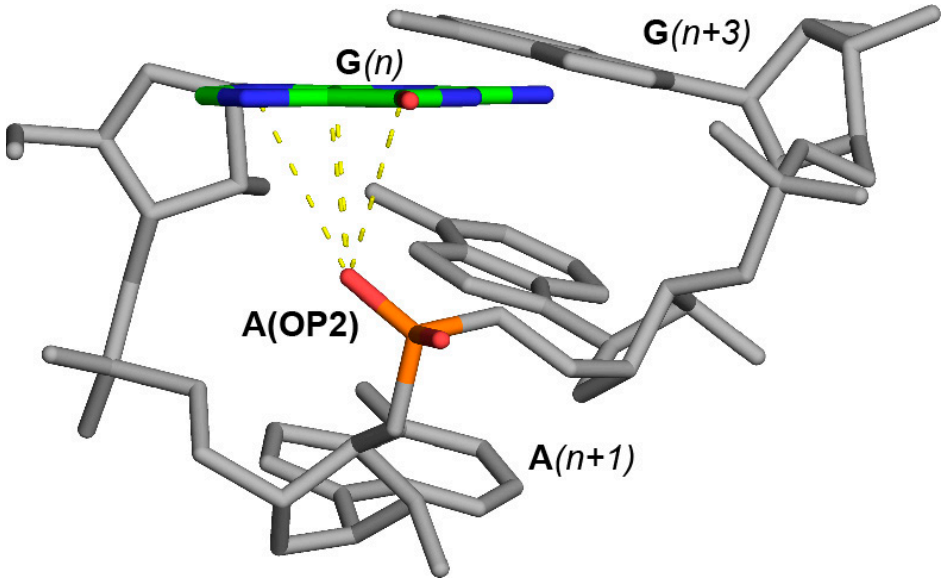
272 C	**Anion-π or cation- π interactions in RNA?**
275 C	**CompaRNA: a server for continuous benchmarking of automated methods for RNA structure prediction**
278 C	**Elucidation of viroids structure by SHAPE**
281 C	**Ligand selectivity of the neomycin RNA aptamer is highly influenced by its ionic surroundings**
284 C	**Crowded Environments Compensate Destabilizing Mutations in the Azoarcus Ribozyme**
287 C	**Crystallization of the active form of the Lariat-Capping ribozyme.**
290 C	**Imp3p unfolds conserved and stable stem structures in both U3 snoRNA and pre-rRNA to promote annealing**
293 C	**Self-dimerizing group I ribozymes as a new class of modular units for RNA synthetic biology**
296 C	**Crystallographic Studies of the Complex between G-quadruplex RNA and the RGG Domain of Fragile X Mental Retardation Protein**

272-C Anion-π or cation-π interactions in RNA?

Pascal Auffinger[1], *Luigi D'Ascenzo*[1], *Eric Westhof*[1]
[1]IBMC/CNRS- UPR9002

In order to better characterize RNA folding and RNA structure as well as RNA interactions with proteins and ligands, it is essential to refine our knowledge of the non-covalent interactions that are at play in these systems. Without doubt hydrogen-bonds are among the best known non-covalent interactions. But, next to them, a large diversity of non-covalent interactions exists. For instance, in the protein world, cation-π interactions, namely the stacking of cationic species over an aromatic group, became rapidly a necessary letter in the non-covalent interaction alphabet. Yet, despite their obvious aromatic character, no significant cation-π interactions were described so far for nucleic acid systems. Here we report that nucleic acid aromatic systems prefer to interact with anionic rather than cationic species.

Indeed, through the calculation of electrostatic potential surfaces, we were able to show that the protein and nucleic acid aromatic systems do not share the same characteristics. The formers are electron-rich systems able to establish cation-π contacts; the latters are electron-depleted systems that display a propensity to attract anions and establish anion-π interactions. This largely unnoticed dissimilarity between aromatic rings of both main biomolecular groups sheds new light on some of their essential properties. Through an exhaustive search of the PDB for anion-π interactions involving, among others, the DNA and RNA backbone phosphate groups, it is found that anion-π interactions are rare in DNA compared to RNA where they are mostly involved in sharp turns (75%) such as those found in tRNA anticodon loops and, more generally, in RNA tetraloops (see Figure 1 below that shows the stacking of a phosphate group under a guanine in a GAAA tetraloop). Besides, these interactions are also observed between sequence-distant residues in ribosomes and in crystal-lattice contacts. The relative rarity of these anion-π interactions outside of loops indicates that it is generally not prevailing over classical hydrogen bonds in the nucleic acid context but nevertheless vital for their folding, structure and function.



275-C CompaRNA: a server for continuous benchmarking of automated methods for RNA structure prediction

Janusz Bujnicki[1], Tomasz Puton[2], Lukasz Kozlowski[3], Sam Mondal[3], Marcin Magnus[3], Kristian Rother[2]

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We have developed a benchmarking approach for the assessment of RNA secondary and tertiary structure prediction methods and implemented it in the CompaRNA web server. In the assessmend of secondary structure prediction methods, he performance of 28 single-sequence and 13 comparative methods has been evaluated on RNA sequences/structures released weekly by the Protein Data Bank. We have also calculated a static benchmark generated on RNA 2D structures derived from the RNAstrand database. Benchmarks on both data sets offer insight into the relative performance of RNA secondary structure prediction methods on RNAs of different size and with respect to different types of structure. The best comparative methods typically outperform the best single-sequence methods if an alignment of homologous RNA sequences is available. The initial tests on RNA 3D structure prediction methods provides additional insight into their strengths and weaknesses, and suggest strategies for the combination of the existing methods into meta-predictors that may aid in constructing superior models.

278-C Elucidation of viroids structure by SHAPE

Tamara Giguère[1], *Charith Raj Adkar-Purushothama*[1], *François Bolduc*[1], *Jean-Pierre Perreault*[1]

[1]Département de biochimie, Université de Sherbrooke, Québec, Canada

Viroids are single stranded, circular RNA with the size range of 246-400 nucleotides. They are the smallest known phytopathogens causing a wide array of symptoms. Viroids do not encode any pathogen-specific proteins and therefore both the secondary as well as the tertiary structures are of the upmost importance. In general, the secondary structures of viroids have been predicted based on their sequence using computer software programs, which have been shown to possess several important limitations. The predicted structure of a viroid needs to receive physical support prior to its use in the accurate interpretation of any mechanistic studies. Recently, we have adapted SHAPE probing coupled with fluorescence sequencing technics and computer-assisted structure prediction in order to fasten the structural determination of viroid species. As proof-of-concept, the secondary structure of the RNA strands of both the plus and minus polarities of the peach latent mosaic viroid has been determined. Both of these structures are in good agreement with the ones obtained using conventional SHAPE protocol. The same methodology has been applied to determine the secondary structure of both the strands (plus/minus) of several viroids of the *Avsunviroidae* family. This easy-to-use and fast protocol will be very useful to compare the structures of many viroid variants revealed from high-throughput sequencing data in a relatively short amount of time.

281-C Ligand selectivity of the neomycin RNA aptamer is highly influenced by its ionic surroundings

Muslum Ilgu[1], Bruce D Fulton[2], Ragothaman Yennamalli[4], Megan M Kleckler[2], Monica H Lamm[2], Taner Z Sen[2], Marit Nilsen-Hamilton[3]

[1]Ames Laboratory, US DOE; [2]Iowa State University; [3]Iowa State University and Ames Laboratory, US DOE; [4]Rice University

Nucleic acid aptamers are frequently characterized as highly specific for their cognate targets, which implies precise molecular recognition between the aptamer and its target. However, as short oligonucleotides, aptamers are likely to be structurally flexible and more responsive to environmental changes adjusting to alternative conformations with altered specificity. To understand the role of aptamer structure in establishing ligand specificity, we examined an RNA aptamer reported as highly selective for neomycin-B over other aminoglycosides. Docking scores correlated well with the experimentally determined dissociation constants showing that the aptamer is more promiscuous than previously reported. The ionic surroundings strongly affected the ligand affinities for its aminoglycoside ligand and influenced the promiscuity of aminoglycoside binding. In parallel, the observation of a flexible pentaloop in molecular dynamics simulations led us to investigate the impact of this region on target selectivity. Studies with aptamer variants, including those with 2-aminopurine substitutions and altered bases, showed that the aptamer conformation was ligand-dependent and strongly affected by the ionic environment. The results of this study demonstrate that the structure of the neomycin aptamer pentaloop is highly malleable, impacted by the ionic environment and coordinating with the binding pocket for ligand incorporation. An impact of buffer composition was also observed on the conformation of the malachite green aptamer interacting with malachite green. Thus, buffer composition may also characterize the interaction of other aptamers with their ligands. These results support a view of aptamer conformations as malleable, responding to the ionic environment and also the ligand structure, with potential impacts on aptamer affinity and specificity.

284-C Crowded Environments Compensate Destabilizing Mutations in the Azoarcus Ribozyme

Hui-Ting Lee[1], Duncan Kilburn[1], Sarah Woodson[1]

[1]Johns Hopkins University, Thomas C. Jenkins Department of Biophysics

Using the *Azoarcus* ribozyme as a model system, our laboratory has previously shown that the native structure of the RNA is stabilized by the cooperative formation of tertiary interactions at different places in the RNA (1). On the other hand, molecular crowding stabilizes the RNA structure (2). Here, we show that crowded environments offset the destabilization due to RNA mutations. We created point mutations in tetraloops (A25U and A190U) or in the central triple helix (G125A) to disrupt tertiary interactions and destabilize the structure. Combinations of these mutations create RNA molecules carrying single, double or triple mutations. We followed the RNA folding process by native PAGE, small angle X-ray scattering (SAXS) and ribozyme activity in the presence of 18% PEG_{1000}. We found that PEG_{1000} reduced the average size of RNA and altered the distribution of unfolded structures at low magnesium concentrations. Furthermore, PEG_{1000} shifted the midpoints of the folding transitions to lower magnesium. In the presence of 18% PEG_{1000}, some mutants even show similar stability as the wild-type ribozyme. The magnitude of this compensation depends on the position of mutation. The results show that crowder molecules in solution compensate for the destabilization caused by mutations, and change the magnitude of cooperativity between tertiary interactions. Greater tolerance to mutation in physiological environments may allow for neutral drift among non-coding RNA sequences.

287-C Crystallization of the active form of the Lariat-Capping ribozyme.

Mélanie Meyer[1], Henrik Nielsen[3], Eric Westhof[2], Steinar Johansen[4], Benoît Masquida[5]

[1]Génétique Moléculaire, Génomique, Microbiologie Université de Strasbourg Institut de Physiologie et Chimie Biologique du CNRS, 21 rue René Descartes F-67084 Strasbourg France; [2]Architecture et Réactivité de l'ARN Université de Strasbourg Institut de biologie moléculaire et cellulaire du CNRS, 15 rue René Descartes F-67084 Strasbourg France; [3]Department of Cellular and Molecular Medecine The Panum Institute University of Copenhagen DK-2200N Copenhagen Denmark; [4]Department of Molecular Biotechnology RNA Research Group Institute of Medical Biology University of Tromsø N-9037 Tromsø Norway; [5]Génétique Moléculaire, Génomique, Microbiologie Université de Strasbourg Institut de Physiologie et Chimie Biologique du CNRS, 21 rue René Descartes F-67084 Strasbourg France

The lariat-capping ribozyme (LCrz) followed by an ORF encoding a homing endonuclease (HE) is embedded in the P2 stem of a regular group I intron named GIR2. This complex twin-ribozyme intron found in the SSU rRNA precursor of several eukaryotes has been mostly studied in *Didymium iridis*. In connection with the microorganism life cycle, three different intron processing pathways are observed. In the regular self-splicing pathway, GIR2 performs splicing to allow the LCrz branching reaction to proceed, leading to a 3' product with the first and the third nucleotide linked by a 2' 5'phosphodiester bound. This three nucleotides lariat caps the HE mRNA and replaces the conventional m^7G cap. LCrz shares high sequence and secondary structure similarities with group I introns, but distinct three-dimensional features. Thus, the LCrz constitutes an independent class of naturally occurring ribozymes. The LCrz branching reaction takes place in a genuine structural context despite the fact it resembles the first step of splicing by group II introns or the spliceosome.

Formerly known as the GIR1 branching ribozyme, it has been renamed regarding the 2' 5' branching reaction it catalyzes and its specific structural features revealed by our 2.5 Å crystal structure of the full-length DiLCrz (192 nucleotides) in the post-cleavage form. We are currently crystallizing the active form of the LCrz to better understand the chemical and structural basis of lariat formation. We now have data up to 4 Å and a readily interpretable electron density map. These data show that the unit cell of the active form of the full-length DiLCrz is shrunk along two dimensions as compared to the post-cleavage form indicating discrete conformational changes with respect to the post-catalytic state.

290-C Imp3p unfolds conserved and stable stem structures in both U3 snoRNA and pre-rRNA to promote annealing

Binal Shah[1], Xin Liu[1], Carl Correll[1]

[1]Rosalind Franklin University of Medicine and Science

Growth of eukaryotic cells depends on ribosome biogenesis and its dysfunction is closely linked to cancer progression. An essential step in making ribosomes is rapid hybridization between the pre-rRNA and the U3 snoRNA, which is required for the pre-rRNA cleavages that liberate the small subunit (SSU) precursor. One of the three prerequisite U3-pre-rRNA hybridization sites, designated the U3-18S duplex, is not observed in vitro in the absence of protein because the bases involved in hybridization in each RNA are buried in conserved and stable structures: box A/A' stem loop in U3 snoRNA and helix 1 (H1) in the 18S region of the pre-rRNA. Thus, energy to unwind these stems is needed to permit hybridization. Previously, we showed that Imp3p and Imp4p are required for U3-18S hybridization in vitro (Gerczei '09). However, the minimal 18S substrate used in that work was unable to form H1 and it was unknown whether protein binding exposes the box A/A' bases. Here, we employ larger pre-rRNA fragments more representative of in vivo substrates. By probing base accessibility with CMCT modification and backbone accessibility with RNase T1 our studies demonstrate that the larger substrates form the secondary structures observed in vivo and that binding of Imp3p alone provides sufficient energy to unfold the pre-rRNA H1 and the U3 box A/A' stem structure. Moreover, this protein dependent unfolding activity is required to observe hybridization. These stable RNA structures may serve as a switch to block U3-pre-rRNA interactions until recruitment of Imp3p, which is expected to happen late in assembly of the 90S preribosome. Imp3p recruitment promotes U3-18S hybridization, which in turn leads to recruitment of the endonucleases that release the SSU precursor.

Gerczei *et al* (2009) *JMB* **390**, 991.

293-C Self-dimerizing group I ribozymes as a new class of modular units for RNA synthetic biology

Takahiro Tanaka[1], *Hiroyuki Furuta*[1], *Yoshiya Ikawa*[1]

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Biopolymers are promising materials for nanotechnology. DNA has been used for the construction of chemically stable 1D, 2D and 3D nano-objects because self-assembly of DNA is easily programmable. On the other hand, polypeptides (proteins) form complex 3D structures and exhibit diverse functions.

From the viewpoint of a nano-biotechnology and synthetic biology, RNA is also an attractive biopolymer. Due to the structural similarity between RNA and DNA, design principles of DNA nano-objects can be applicable to the design of RNA nano-objects. Moreover, RNA is also able to perform protein-like functions such as catalysis and molecular recognition because RNA can also form complex 3D structures. Several RNA nano-structures such as polygons and polyhedrons have been reported but they were composed of relatively short and simple RNA units (ca. 25-100 nts). In contrast, functional RNAs in living cells such as ribosomes and self-splicing ribozymes are composed of 200-3000 nucleotides.

We have constructed a new class of nano-structures possessing catalytic activity derived from a group I intron ribozyme. Because RNA nano-structures possessing the enzyme-like functions has not been reported so far. We converted a group I intron ribozyme to self- or hetero-dimerizing RNA that exhibits enzyme function upon self-dimerization. Molecular design for the dimerization was carried out with modular engineering, by which intramolecular interactions of the parent ribozyme was redesigned to form intermolecular interactions for the dimerization. The dimerization proceeded highly effectively and the ribozyme activity was strongly depended on the RNA dimerization. The resulting dimeric ribozyme would be applicable to new device modules for RNA synthetic biology.

296-C Crystallographic Studies of the Complex between G-quadruplex RNA and the RGG Domain of Fragile X Mental Retardation Protein

Nikita Vasilyev[1], Anna Polonskaia[2], Dinshaw J. Patel[2], Alexander Serganov[1]

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Fragile X syndrome is one of the leading causes of inherited mental retardation and autism. The syndrome is associated with the loss of functional Fragile X Mental Retardation Protein (FMRP). In neurons, FMRP binds to a subset of neuronal mRNAs, normally activated by specific receptors, and inhibits translation of these mRNAs. FMRP contains three canonical RNA-binding domains: two KH domains and an arginine-glycine rich (RGG) box. The RGG motif recognizes mRNAs that contain guanine-rich regions capable of forming guanine quartets. We have determined the crystal structure of the complex between the RGG peptide and a guanine-rich RNA at 2.8 Å resolution. The RNA structure revealed formation of three G-quartets assembled in a potassium-stabilized G-quadruplex and connected to a helical stem. The RGG peptide interacts predominantly with the stem and the adjacent junction through base-paring with guanine bases and the sugar-phosphate backbone. The structure explains previous biochemical observations and suggests the molecular principles of FMRP binding to natural mRNAs.

RNA chemistry

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 299 C – 305 C
Location: Main Hallway & Sanada Foyer

299 C	**Spin-labeled cytidine nucleotides in long synthetic RNAs: solid-phase synthesis, post-synthetic labeling, ligation and EPR-spectroscopic characterization**
302 C	**Targeting secondary structures of pre-miRNA by small molecules: Development of potential inhibitors of pre-miRNA processing**
305 C	**Coumarin fluorochrome binds to Rev responsible element RNA with extremely large absorption shift**

299-C Spin-labeled cytidine nucleotides in long synthetic RNAs: solid-phase synthesis, post-synthetic labeling, ligation and EPR-spectroscopic characterization

Claudia Höbartner[1], Jan Seikowski[1], Lea Büttner[1], Katarzyna Wawrzyniak[1], Anne Ochmann[1], Falk Wachowius[1], Giuseppe Sicoli[2]

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Chemically stable nitroxide radicals that can be monitored by electron paramagnetic resonance (EPR) spectroscopy can provide information on structural and dynamic properties of functional RNAs, such as for example riboswitches. Since natural RNA is diamagnetic, the site-specific installation of nitroxide spin-labels is required. A number of post-synthetic strategies have been established for the attachment of nitroxide spin labels at various sites in pre-functionalized RNA, but advanced EPR techniques and instrumentation demand the development of new spin-labeling approaches for RNA. Challenges that need to be addressed include the synthesis of rigid spin labels and the preparation of long spin-labeled RNA by combined chemical and enzymatic strategies.

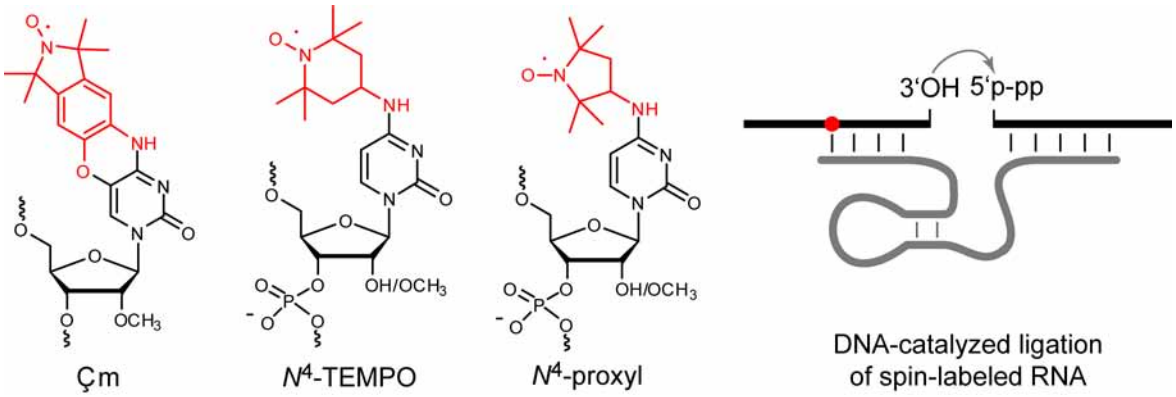
We highlight the synthesis and application of the nitroxide-containing nucleoside C¸m, reported as the first rigid spin label for paramagnetic modification of RNA by solid-phase synthesis[1,2] and the convertible nucleoside approach that enabled the direct attachment of TEMPO and proxyl spin labels at the exocyclic N^4-amino group of cytidine and 2'-*O*-methylcytidine nucleotides in RNA.[3,4] Recent results on the synthesis of long spin-labeled RNAs will be discussed, based on using deoxyribozymes as alternatives to protein enzymes for ligation of spin-labeled RNA. To obtain site-specifically labeled long riboswitch RNAs beyond the limit of solid-phase synthesis, we report the ligation of paramagnetic RNA using an in vitro selected deoxyribozyme as catalyst, and demonstrate the synthesis of TEMPO-labeled 53-nt SAM-III and 118-nt SAM-I riboswitch domains (SAM = *S*-adenosylmethionine).[4]

[1] C. Höbartner, G. Sicoli, F. Wachowius, D.B. Gophane, S.T. Sigurdsson, Synthesis and characterization of RNA containing a rigid and nonperturbing cytidine-derived spin label, *J. Org. Chem.* **2012**, *77*, 7749.

[2] I. Tkach, S. Pornsuwan, C. Höbartner, F. Wachowius, S.T. Sigurdsson, T. Baranova, U. Diederichsen, G. Sicoli, M. Bennati, 'Orientation selection in distance measurements between nitroxide spin labels at 94 GHz EPR with variable dual frequency irradiation', *Phys. Chem. Chem. Phys.* **2013**, *15*, 3433.

[3] G. Sicoli, F. Wachowius, M. Bennati, C. Höbartner, Probing secondary structures of spin-labeled RNA by pulsed EPR spectroscopy, *Angew. Chem. Int. Ed.* **2010**, *49*, 6443.

[4] L. BÜttner, J. Seikowski, K. Wawrzyniak, A. Ochmann, C. Höbartner, **2013**, submitted.



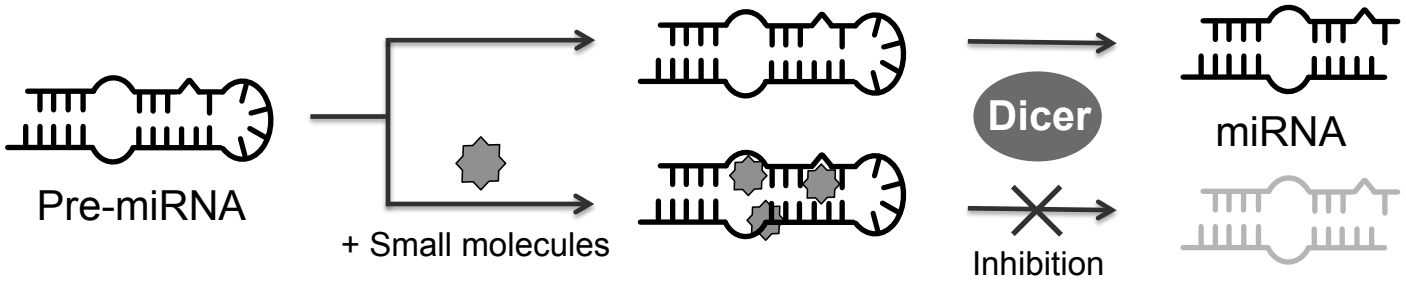
302-C Targeting secondary structures of pre-miRNA by small molecules: Development of potential inhibitors of pre-miRNA processing

Asako Murata[1], Ayako Sugai[1], Takeo Fukuzumi[1], Shiori Umemoto[1], Chikara Dohno[1], Kazuhiko Nakatani[1]

[1]Osaka University

MicroRNAs (miRNA) are involved in many biological processes including development, differentiation and carcinogenesis through translational repression by binding to a target mRNA. Inhibition of miRNA pathways by altering miRNA expression and/or maturation in cells would modulate gene expression, and enable us to understand miRNA regulatory effects on various biological processes. Anti-miRNA oligonucleotide (antagomir) is the most readily available tool to knock-down the expression of an endogenous miRNA, and thereby perturb miRNA-mediated gene regulation in a sequence specific manner. In addition to the antagomir approach, a small molecule that bind to precursor-miRNA (pre-miRNA) and inhibit Dicer-catalyzed pre-miRNA processing will provide other options for modulating miRNA-mediated gene regulation. Several groups have shown that small molecules such as aminoglycosides, peptides, and peptoids would be potential inhibitors of Dicer-catalyzed pre-miRNA processing.

We have previously reported the synthesis and structure-activity relationships of xanthone and thioxanthone derivatives as the fluorescent indicators for detecting the interactions between RNA and small molecules [*J. Am. Chem. Soc.* **2010**, 132, 3660., *Chem. Eur. J.* **2012**, 18, 9999.]. Some of the 2,7-disubstituted xanthone and thioxanthone derivatives preferentially bind to certain secondary structures of RNA such as loops and bulges rather than double-stranded regions. Since most pre-miRNAs have such secondary structures, we explored a possibility of inhibitory activity of the xanthone and thioxanthone derivatives against the dicing reaction upon their binding to pre-miRNA. We herein report that an aminoalkoxy-substituted thioxanthone derivative interferes Dicer-mediated processing of pre-miRNA (the figure below). Chemically synthesized pre-miR-29a was digested by human recombinant dicer in the absence or presence of xanthone and thioxanthone deriviative, and the resulting products were analyzed by denaturing PAGE. X2SS, one of the thioxhanthone derivatives we synthesized, effectively suppressed the formation of both the intermediate and mature miR-29a, indicating the inhibitory effect of X2SS on pre-miR-29a processing. Analysis of the dicing reaction of pre-miR-29a mutant revealed that binding of X2SS close to a cleavage site is capable of interfering the processing of pre-miR-29a. Information about the interaction between these xanthone derivatives and pre-miRNAs will enable us to design and develop new small molecule-based inhibitors for miRNA pathway.



305-C Coumarin fluorochrome binds to Rev responsible element RNA with extremely large absorption shift

Tetsuya Tsuda[1], *Takeo Fukuzumi*[1], *Kazuhiko Nakatani*[1]

[1]Osaka University

Since the discovery of a role for RNA in modulating gene expression, functional RNA has become an attractive drug target. However, there is not much report of the synthetic molecules that bind to RNA. Because of the complicated conformation of RNA, it is difficult to design binding molecules only by the conventional molecular design. The development of ligands that bind selectively to a particular structure and sequence of RNA is useful to understand and modulate the function of RNA. In this study, we focused on the design and synthesis of novel ccoumarin derivatives that can bind to particular secondary structures of RNA. It is well known that C7-substituted coumarin derivatives with electron-donating group at the C7 position show strong fluorescence. In addition, further substitution with a electron-accepting group at the C3 position change their electron spectrum, which are characterized by such as red shift of absorption maximum and increase of fluorescence intensity. Considering these characters of coumarin, we envisioned that modification of coumarin at C3 and C7 positions enable us to observe binding of coumarin derivatives to RNA as a change in electronic spectrum.

We designed and synthesized a series of coumarin derivatives, in which piperazinyl group at the C7 position is introduced as a electron-donating group. The piperazinyl group would facilitate binding to RNA, due to its positive charge. Various functional groups, such as acetyl and benzoyl group, pyridine analogues, benzimidazole analogues, and 1,8-naphthyridine analogues is introduced to coumarin at the C3 position. Binding affinity of these coumarin derivatives to RNA were investigated by the absorption and fluorescence spectra in the presence of RNA. We used Rev responsible element (RRE), as the model RNA that consist of an internal loop and a terminal loop, and double-strand regions. 7-Piperadinylcoumarin, a control compound that does not have any functional group at the C3 position, showed only a small change of electronic spectrum when titrated with RRE. In contrast, apparent large red shift of absorption maximum from 398 nm to 460 nm was observed in the coumarin derivative having 2'-aminonaphthyridine at the C3 position (**TT7**) with one equivalent of RRE. To investigate the binding site of **TT7** to RRE, we prepared three RRE-mutants. RRE-mutant 1 lacked both the internal and hairpin loops of RRE, RRE-mutant 2 lacked the hairpin loop, and RRE-mutants 3 lacked the internal loop. Large red shift of absorption maximum was also observed when **TT7** was titrated with RRE-mutant 2 or 3. These results suggested that **TT7** bound selectively to the hairpin loop and changed to absorption spectrum.

Therapeutic RNAs

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 308 C – 314 C
Location: Main Hallway & Sanada Foyer

308 C	**ANTISENSE RNA-INDUCED EXON-SKIPPING FOR THE GENE THERAPY OF FRONTOTEMPORAL DEMENTIA AND PARKINSONISM ASSOCIATED WITH CHROMOSOME 17 (FTDP-17)**
314 C	**Combined systemic and local morpholino treatment rescues the phenotype of the SMA Delta 7 mouse model**

308-C ANTISENSE RNA-INDUCED EXON-SKIPPING FOR THE GENE THERAPY OF FRONTOTEMPORAL DEMENTIA AND PARKINSONISM ASSOCIATED WITH CHROMOSOME 17 (FTDP-17).

Giuseppina Covello[1], Kavitha Siva[1], Lara Mari[3], Elena Marchesi Marchesi[3], Perrone Daniela Perrone[3], Michela Alessandra Denti[2]

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Tau, encoded by a single gene on chromosome 17p21, is a cytoskeletal component expressed in the central nervous system (CNS), with a role in neurogenesis, axonal maintenance and axonal transport. A number of neurodegenerative diseases, including FTDP-17 (rare autosomal dominant condition), are characterized by intra-neuronal accumulation of the tau protein.

The genetic linkage between tau protein and FTDP-17 seems to be, for about half of the cases of FTDP-17, mutations affecting the alternative splicing of exon 10 (E10) of the tau mRNA. The aberrant inclusion of E10 in the tau mRNA, in fact, leads to the aggregation of tau as Neurofibrillary Tangles (NFTs) in brain

The project explores the feasibility of an antisense (as-) RNA-based gene therapy to correct tau splicing in FTDP-17.

We first tested whether it was possible to modulate E10 alternative splicing by the usage of Antisense Oligo-Nucleotides (AONs) that mask specific sites regulating splicing pathways.

RT-PCR and Western blot analyses showed that the transfection of specific AONs is able to alter the splicing behaviour of tau E10 in the rat endogenous transcript (PC-12 cell lines), with variable efficiencies depending on the concentration of the AONs and on the targeted sequence. The results were confirmed by transfection of Scramble Control Oligonucleotides that had no effects on E10 inclusion at the same concentration.

Based on these results, we constructed Adeno-Associated Viral (AAV) vectors coding for specific as-RNAs. We embedded the as-RNA sequences in chimeric U snRNA vectors whose promoters themselves lead to long-term as-RNA expression. We tested whether, the splicing behaviour of tau is corrected in endogenous rat mRNA by these chimeric antisense sn-RNAs.

To evaluate the effects of AONs/Chimeric Antisense-snRNA on the human tau pre-mRNA, we constructed a minigene reporter system, containing luciferase and that recapitulates to a large extent the behaviour of E10 in the context of the full-length tau gene. We carried out co-transfection into HeLa cells and evaluated the induction of E10 skipping by Luciferase Expression Assay, Real Time-PCR and Western blot analyses. Further work will be directed to test the therapeutic efficacy of the AONs and AAV-vectored as-RNAs in the animal model of FTDP-17 (T-279 mouse).

This project was supported by TELETHON Italia Grant GGP08244

314-C Combined systemic and local morpholino treatment rescues the phenotype of the SMA Delta 7 mouse model

Marc-David Ruepp[1], Monica Nizzardo[3], Chiara Simone[3], Sabrina Salani[3], Federica Rizzo[3], Margherita Ruggieri[3], Simona Brajkovic[3], Hong Moulton[2], Oliver Mühlemann[1], Nereo Bresolin[3], Giacomo P. Comi[3], Stefania Corti[3]

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Spinal muscular atrophy (SMA) is a childhood fatal motor neuron disease caused by mutations in the Survival Motor Neuron 1 (SMN1) gene, currently without effective treatment. One possible therapeutic approach is the use of antisense oligonucleotides (ASOs) to redirect the splicing of a paralogous gene, SMN2, to increase the production of functional SMN protein. A range of ASOs with different chemical properties is suitable for these applications, including a morpholino (MO) variant, which has a particularly excellent safety, and efficacy profile. We used a 25-nt MO oligomer sequence against the ISS-N1 region of SMN2 (HSMN2Ex7D(-10-34)) with superior efficacy to previously described sequences also in transgenic SMA ?7 mice. The combined local and systemic administration of MO (bare or conjugated to octa-guanidine) is necessary to increase full-length SMN expression, leading to robust neuropathological features improvement and survival rescue. Additionally, several snRNA levels that are dysregulated in SMA mice could be restored by MO treatment. These results demonstrate that MO therapy is efficacious and can result in phenotypic rescue. These data provide important insights for the development of therapeutic strategies in SMA patients.

Workshop: RNA in pharmaceutical research

Date: Saturday, June 15, 14:00 - 17:00
Abstract: 311 C
Location: Main Hallway & Sanada Foyer

311 C **Inhibition of the HIV -1 virus RRE-Rev interaction by small molecule Rev mimics. A new synthetic scaffold for specific recognition of RNA structure.**

311-C Inhibition of the HIV -1 virus RRE-Rev interaction by small molecule Rev mimics. A new synthetic scaffold for specific recognition of RNA structure.

Luis Gonzalez-Bulnes[1], Ignacio Ibañez[4], Luis Miguel Bedoya[2], Silvia Catalan[3], Jose Alcami[2], Santos Fustero[3], Jose Gallego[1]

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Functional and structured RNA motifs are not easily targeted by antisense agents and have the advantage of their high sequence and/or three-dimensional structure conservation. However, leaving aside natural products binding to sites within bacterial ribosomal RNA, the development of small RNA-binding agents has been hampered by the difficulties posed by these structures, which have limited physicochemical diversity and are often flexible. In order for this approach to be successful, it is essential to identify novel chemical scaffolds capable of specifically recognizing these motifs1. The Rev Recognition Element (RRE) is a strongly conserved 350 -nucleotide structure located in the *env* gene of human immunodeficiency virus type-1 (HIV-1) RNA. Within subdomain IIB of the RRE, the unusually widened major groove of a large 5:6 internal loop forms a high-affinity complex with the arginine-rich a-helix of the virally-encoded protein Rev, Rev34 -50. This interaction is essential for virus viability, as it triggers a cascade of events allowing the transport of unspliced or incompletely spliced viral RNA molecules to the cytoplasm of the infected cell. The RRE-Rev system offers unique advantages for drug design: the three-dimensional structures of the RRE-Rev34-50 complex and of unbound RRE and Rev are known. Making use of these advantages, we used structure-based methods to design small molecule mimics of Rev with a novel chemical scaffold. A set of these ligands was subsequently synthesized and tested *in vitro* and *in vivo*. Surface plasmon resonance and isothermal titration calorimetry experiments indicated that the compounds bound specifically to the RRE with an affinity of 5 μM. Nuclear magnetic resonance spectroscopy analyses showed that they occupied the binding site of Rev34 -50 in the major groove of the IIB loop, inducing an RNA conformational change strickingly similar to that detected upon Rev34-50 binding. Fluorescence polarization experiments indicated that these terphenyls were capable of inhibiting the RRE-Rev34 -50 interaction *in vitro.* Cellular assays demonstrated that the most potent inhibitors blocked HIV -1 replication *in vivo* and exerted this effect post- transcriptionally, as expected2. Most of the small RNA-binding agents described so far are related to peptides or antibiotics, or were discovered by screening. To our knowledge, this is the first time that a new organic scaffold with RNA-based activity has been designed *de novo.*

1. Gallego, J. & Varani, G. Targeting RNA with Small Molecule Drugs: Therapeutic Promise and Chemical Challenges. *Acc. Chem. Res.* **34**, 836 -843 (2001).

2. Gonzalez-Bulnes, L.; Ibañez, I.; Bedoya, L.M.; Catalan, S.; Alcami, J.; Fustero, S.; Gallego, J. Patent application P201330235, 21-2-2013.

tRNA, snRNA, snoRNA, rRNA

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 317 C – 332 C
Location: Main Hallway & Sanada Foyer

317 C	**Lateral tRNA gene transfer in Methanobrevibacter ruminantium**
320 C	**Gene expression analysis of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code**
323 C	**Strong anion exchange fast performance liquid chromatography as a versatile tool for preparation and purification of RNA produced by in vitro transcription**
326 C	**RIP-seq analysis of eukaryotic Sm proteins reveals interactions between snRNPs and mature mRNAs**
329 C	**A comprehensive analysis of the natural variation of tRNA modification in two Saccharomyces species**
332 C	**Playing RNase P evolution: replacing a complex ribonucleoprotein enzyme with a single protein**

317-C Lateral tRNA gene transfer in Methanobrevibacter ruminantium

Patricia Chan[1], *Todd Lowe*[1]

[1]University of California Santa Cruz

The increase of the available microbial genomes has enabled thorough comparative studies across multiple species. Substantial differences in gene content among closely related species in addition to the similarities found among distant species provide evidence of genetic information exchange. One well-known example is *Thermotoga maritima*, a thermophilic bacteria reported to contain 24% of genes that are homologs from archaea [1]. While multiple possible mechanisms may involve in the process of lateral gene transfer, most research have been solely focused on examining the origin of protein-coding genes. The extent of non-coding RNA gene transfer across species remains unclear.

During our study of *Methanobrevibacter ruminantium*, an archaeon that was isolated from bovine rumen fluid, we identified 59 tRNA genes as compared to 36 in its close relative, *Methanobrevibacter smithii*. A detailed inspection shows that 20 of these tRNA genes have a second copy with different sequence but same anticodon. While a small number of archaeal tRNAs has two copies in a genome, over 90% of them are single-copy genes. More unexpectedly, a copy of these 20 tRNA genes was found in a cluster within a region of 4000 bp. This represents about four times the size of the largest archaeal tRNA gene cluster. In addition, a tRNA-ArgACG that has never been found in archaea [2] was also identified in this cluster. Another round of gene prediction using bacterial tRNA model suggests that these 21 tRNA genes may have a bacterial origin. The 13% of bacterial homologs reported in a previous study [3] and some found upstream of the tRNA cluster further confirm the foreign insertion of this region.

Lateral gene transfer has been considered as a powerful evolutionary force for microbial genomes. However, the reasons and the biological significance of this event are not fully understood. Our finding demonstrates the first example of tRNA gene transfer between archaea and bacteria and highlights opportunities for new RNA biology.

[1] Nelson et al. (1999) *Nature* 399:323-9.

[2] Grojean et al. (2007) *Nucleic Acids Symp Ser (Oxf)*:15-6.

[3] Leahy et al. (2010) *PLoS One* 5:e8926.

320-C Gene expression analysis of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code

Kiyofumi Hamashima[1], *Yoshiki Andachi*[2], *Masaru Tomita*[1], *Yuji Kohara*[2], *Akio Kanai*[1]

[1]Institute for Advanced Biosciences, Keio University; [2]Genome Biology Laboratory, Center for Genetic Resource Information, National Institute of Genetics, Research Organization of Information and Systems

Transfer RNAs (tRNAs) are small RNA molecules that play a crucial role in protein biosynthesis as the links between the codons and the amino acids. Although translational fidelity is essential for protein and cell integrity, which is achieved by accurate tRNA aminoacylation, we have previously found unexpected nematode tRNAs (nev-tRNAs) that possess a glycine or isoleucine anticodon but can be charged with leucine (1). An *in vitro* translation analysis showed that nev-tRNAs can be incorporated into eukaryotic ribosomes and participate in protein biosynthesis, indicating that nev-tRNAs decode an alternative genetic code for leucine at least *in vitro*.

To understand the biological function of these unusual tRNAs, we first performed gene expression analyses of nev-tRNAs in *Caenorhabditis elegans* and *C. brenneri*, which has more number of nev-tRNAs than any other nematodes. The expression of nev-tRNA^{Gly} (CCC) and nev-tRNA^{Ile} (UAU) genes in mixed stage (egg, larvae 1-4, adult) of both species was detected by RT-PCR and northern blot analysis, but the levels are quite lower than those of general tRNAs. Further expression analysis during nematodes development and the results showed that the expression levels in egg and adult stages are higher than those of other stages. Taken together, our findings suggest that the expression of nev-tRNAs might be generally down-regulated, but stimulated in specific conditions and play a certain role, for example, in development. Now, we are constructing an over-expression system of heat-inducible nev-tRNA genes in *C. elegans* for multi-omics approach. Possible functions of this RNA molecule are discussed.

1. Hamashima, K. et al., (2012) *Nucleic Acids Res*, **40**, 3653-3662.

323-C Strong anion exchange fast performance liquid chromatography as a versatile tool for preparation and purification of RNA produced by in vitro transcription

Jiri Koubek[1], Ku Feng Lin[5], Yet Ran Chen[2], Richard Ping Cheng[3], Joseph Jen Tse Huang[4]

[1]Chemical Biology and Molecular Biophysics Program, Taiwan International Graduate Program, Institute of Chemistry, Academia Sinica, No.128, Sec. 2, Academia Road, Nankang, Taipei 11529, Taiwan; Department of Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan; [2]Agricultural Biotechnology Research Center, Academia Sinica, No.128, Sec. 2, Academia Road, Nankang, Taipei 11529, Taiwan; [3]Department of Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan.; [4]Institute of Chemistry, Academia Sinica, No.128, Sec. 2, Academia Road, Nankang, Taipei 11529, Taiwan; "5Institute of Chemistry, Academia Sinica, No.128, Sec. 2, Academia Road, Nankang, Taipei 11529, Taiwan

In vitro transcription is a common technique for producing large quantities of RNAthat requires exquisite control of a number of variables including template preparations, enzyme selection, transcription reaction conditions and purification protocols.

Here we demonstrate the use of strong anion exchange fast performance liquid chromatography (FPLC) as a simple, fast and robust technique for RNA production by *in vitro* transcription. With this method, we purified short transcription templates from unreacted reagents in large quantities; only minor changes to the protocol were necessary to readily obtain nuclease free pyrophosphatase. In addition, *in vitro* transcription reactions were monitored by strong anion exchange FPLC to enable facile optimization of reaction conditions. Transcribed tRNA was purified by strong anion exchange FPLC and the functionality of purified tRNA was confirmed by enzymatic assay. Every procedure described here required only minimum sample manipulation and was completed within 30 minutes.

326-C RIP-seq analysis of eukaryotic Sm proteins reveals interactions between snRNPs and mature mRNAs

Zhipeng Lu[1], *Xiaojun Guan*[2], *Greg Matera*[1]

[1]Department of Biology, UNC at Chapel Hill; [2]Sequenom, San Diago, California

Sm proteins are a family of highly conserved RNA-binding factors present in all three domains of life. Sm proteins usually form hexameric or heptameric rings and associate with a variety of RNAs, and these RNP complexes perform many important roles in RNA metabolism. In bacteria, the Sm ortholog (called Hfq) associates with small (s)RNAs to regulate target mRNA stability and translation. In eukaryotes, Sm proteins bind small nuclear RNAs (snRNAs) to form snRNPs, which are basic components of the pre-mRNA splicing machinery. However, little is known about other functions of Sm proteins in eukaryotic cells, given their divergent roles in bacteria and archaea. Our lab recently found that Sm proteins are required for germ cell specification in *Drosophila*. This discovery led us to hypothesize that Sm proteins play important, but so far unrecognized, roles in RNA metabolism and fundamental cellular processes in eukaryotes.

To test this hypothesis, we developed a strategy to discover novel RNPs by deep sequencing immunopurified RNAs (RIP-seq) that associate with several distinct Sm proteins in *Drosophila* ovaries. Using this approach, we discovered, in addition to the known snRNAs, a highly reproducible subset of Sm-associated mRNAs and several novel, unannotated non-coding RNAs. One of the newly identified ncRNA is a bona fide snRNA, with a clearly recognizable Sm binding site and an snRNA-like secondary structure. We showed that the association between mRNAs and Sm proteins is independent of splicing, and distinct from the LSm1-7-mRNA interaction, which regulates mRNA degradation. Many of the Sm-associated mRNAs encode mitochondrial and ribosomal/translation-related proteins. We further verified these Sm-associated RNAs using RT-PCR on several different tissue/cell types.

Several lines of evidence suggest that Sm proteins associate with mRNAs indirectly. To characterize these RNP complexes, we performed immunoprecipitations with an anti-TMG cap antibody. Together with publicly available RIP-seq data targeting U1-70K (a U1 snRNP protein), we showed that Sm-associated mRNAs also co-purify with snRNAs and snRNP-specific proteins. Correspondingly, we identified potential snRNA base pairing interactions within these Sm-associated mRNAs. These data suggest that snRNPs interact with mature mRNAs directly through base pairing, raising an interesting similarity to the prokaryotic Sm (Hfq)-containing sRNPs.

To test whether this snRNP-mature mRNA interaction is conserved in evolution, we performed Sm RIP-seq in human HeLa cells. In addition to the spliceosomal snRNAs, we identified one of the histone mRNAs as highly enriched, confirming a previous report on U2 snRNP - histone mRNA interaction (Friend et al., 2007, Mol. Cell). We also identified many other mature human mRNAs associated with Sm proteins, suggesting that the snRNP-mature mRNA interaction is conserved in eukaryotes.

329-C A comprehensive analysis of the natural variation of tRNA modification in two Saccharomyces species

Peter Sarin[1], *Sebastian Leidel*[1]

[1]Max Planck Institute for Molecular Biomedicine

Transfer RNA (tRNA) is essential for protein synthesis by linking messenger RNA (mRNA) codons to the respective peptide sequence of proteins. Notably, tRNA molecules are decorated by a plethora of chemical modifications of their nucleobases or sugar backbone. Many of these RNA modifications are conserved throughout evolution, implying their importance. However, little is known about their *in vivo* function and whether RNA modifications are regulated. Furthermore, to date there has not been a single comprehensive study that analyzes the variation of tRNA modification in any species at the population level.

During evolution, yeast has adapted to grow in many ecological niches enabling it to cope with a wide range of external stress factors. This has led to a specific adaptation of cellular metabolism to very specific types of stress in different yeast strains. To elucidate how tRNA modification systems have evolved to modulate cellular adaptation to stress, we set out to analyze modified tRNA nucleosides in a large set of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* strains isolated from various environmental habitats. By means of two-dimensional thin layer chromatography (TLC) we have performed tentative characterization of basal level tRNA modification at logarithmic growth conditions for a total of 73 *Saccharomyces* strains.

Our preliminary screen shows variance in tRNA modification, allowing us to cluster strains according to their tRNA modification levels. We observed considerable variability of tRNA modification levels in *S. cerevisiae* strains. Surprisingly, this variability was much lower in *S. paradoxus* although the genetic diversity of the strains used in our analysis was larger than that of *S. cerevisiae*. Furthermore, mapping the strains according to single nucleotide polymorphisms (SNPs) in tRNA modification genes yields a similar clustering. This might imply, that key SNPs are essential in modulating basal level tRNA modification.

We are currently verifying our analysis using high performance liquid chromatography (HPLC) coupled with mass spectrometry to define the first comprehensive, quantitative high-resolution inventory of tRNA modification in representative *Saccharomyces* populations. Utilizing this approach will allow us to generate the first species wide analysis of tRNA modification.

332-C Playing RNase P evolution: replacing a complex ribonucleoprotein enzyme with a single protein

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RNase P is the endonuclease that removes 5' extensions from tRNA precursors. Despite their uniform and rather simple functional role, members of the RNase P enzyme family are of exceptional diversity. In their more ancient and widespread form, RNase P enzymes are ribonucleoproteins (RNP) based on a structurally conserved RNA molecule forming their catalytic core. In bacteria, a single small protein associates with the catalytic RNA; in the nucleus of some Eukarya (e.g., animals and fungi), however, the RNP reaches considerable complexity by including a set of up to 10 different proteins. A fundamentally different form of RNase P is composed of a single 60-kDa protein termed "proteinaceous" RNase P (PRORP) and is restricted to Eukarya. First found in mitochondria and chloroplasts, PRORPs were recently also found to be responsible for nuclear tRNA maturation in plants and trypanosomatids. In contrast to the complexity of the RNP form of nuclear RNase P, PRORPs have generally remained "simple", with the notable exception of animal mitochondrial RNase P, which is a multi-enzyme assemblage.

The evolutionary constraints and driving forces underlying this bewildering diversity of RNase P enzymes remain largely obscure. Increased enzyme versatility with respect to substrate range was suggested to be a possible reason for the complexity of the nuclear RNP-form of the enzyme. However, the finding that some organisms use a single protein for nuclear RNase P function challenges this view and suggests that either a single protein is as versatile as a complex RNP, or the spectrum of biological functions of the two enzyme forms in their host organisms is different.

By genome engineering, we replaced yeast nuclear RNase P with PRORP3 derived from *A. thaliana*. A thorough phenotypical characterization of different RNase P-swapped strains did not reveal any fundamental difference in the ability to grow under diverse environmental conditions or in their overall fitness. This indicates that a single protein is able to fully fill the biological role of nuclear RNase P in yeast, which naturally is a 10-component RNP. Molecular analyses moreover revealed normal levels of tRNAs and no evidence for alterations in a variety of suggested non-tRNA substrates that were previously reported to accumulate in an RNase P-deficiency model. Our results suggest that there is no inherent need for or advantage of RNA-based catalysis or enzyme complexity to cope with (nuclear) RNase P function.

Ribosomes and Translation

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 335 C – 359 C
Location: Main Hallway & Sanada Foyer

335 C	**Expanded Function of Trans-Editing Domains To Edit Non-canonical Amino Acids Prevents Errors In Translation**
338 C	**Exploring translation in S. pombe using ribosomal profiling**
341 C	**High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome**
344 C	**Interactions of modified oligonucleotides with RNA of the prokaryotic and eukaryotic decoding site**
347 C	**Rrp5 binds the pre-rRNA at multiple sites and is required for ribosomal processing and assembly**
350 C	**Evolutionary decline for a nuclear-encoded human mitochondrial aminoacyl-tRNA synthetase**
353 C	**Non-ribosomal Interaction Partners of Ribosomal Protein S3**
356 C	**Investigating the function of the RNA helicase Prp43 and its cofactor Pfa1 in 40S ribosomal subunit synthesis**
359 C	**Going through the motions: Network analysis reveals conserved intramolecular communication pathways within EF-Tu responsible for ribosome dependent GTPase activation and nucleotide exchange**

335-C Expanded Function of Trans-Editing Domains To Edit Non-canonical Amino Acids Prevents Errors In Translation

Jo Marie Bacusmo[1], Birgit Alber[2], Karin Musier-Forsyth[1]

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Aminoacyl-tRNA synthetases (ARSs) activate specific amino acids and attach them to cognate tRNAs for use in protein synthesis. ARSs often misactivate isosteric standard and non-proteinaceous amino acids, potentially resulting in errors in translation that could be detrimental to cell survival. In the case of prolyl-tRNA synthetases (ProRS), smaller Ala and similar sized Cys are misactivated together with non-standard amino acids 4-hydroxyproline, azetidine, and a-aminobutyrate (Abu). Thus, due to ProRS promiscuity, editing mechanisms have evolved to ensure fidelity in Pro codon translation. In many bacterial systems, a so-called "triple-sieve" editing mechanism is employed, which consists of the ProRS active site that discriminates amino acids based primarily on volume and size (i.e., the "coarse sieve"), the ProRS editing domain (INS) that hydrolyzes Ala-tRNA^{Pro} in *cis* (i.e., the "fine sieve"), and a single-domain INS homolog YbaK that clears Cys-tRNA^{Pro} in *trans*. The latter occurs via a novel mechanism involving substrate sulfhydryl side-chain chemistry (i.e., the "chemical sieve"). Although these mechanisms clear standard non-cognate amino acids, how non-canonical amino acids are prevented from misincorporation is unclear. Moreover, many different combinations of INS-like *cis* and *trans*-editing domains exist in bacteria. For example, the metabolically versatile bacterium *Rhodopseudomonas palustris* (Rp) encodes a ProRS containing a truncated INS domain that we have shown is catalytically inactive, in addition to two distinct, INS homologs YbaK and ProXp-x. The function of the latter is unknown. In this work, Rp ProXp-x was cloned and purified and shown to have only weak activity in preventing mischarging of Ala-tRNA^{Pro} and Cys-tRNA^{Pro} *in vitro*. Comparison of known crystal structures reveals that the putative substrate-binding pocket of ProXp-x is larger than that of INS, which suggests substrates larger than Ala are preferred. Indeed, we demonstrate here that ProXp-x shows robust editing of the non-canonical amino acid Abu, a metabolite involved in catabolic and metabolic pathways of numerous amino acids to which ARSs are exposed. Rp ProRS specificity for activation of Pro over Abu is only about 1,000:1, which strongly suggests that editing is required *in vivo*. Furthermore, Abu is mischarged onto tRNAs not only by ProRS, but also by ValRS and IleRS and ProXp-x also displays robust editing of Abu-tRNA^{Val}. Taken together, these data suggest that Abu-tRNA editing by the *trans*-editing factor ProXp-x is likely to be a critical checkpoint to ensure high fidelity in codon translation.

338-C Exploring translation in S. pombe using ribosomal profiling

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Ribosomal profiling is a powerful technique to measure translation genome-wide in growing cells. By comparing ribosome protected fragments to mRNA abundance, translation efficiency can be calculated for every translated mRNA. *Schizosaccharomyces pombe* has been extensively characterized at the mRNA level; however, much less is known about translational control. To address this question we have performed ribosomal profiling of a wild type *S. pombe* strain. Analysis of our data has allowed the identification of translation start sites, uORFs, potential frameshifting events and translation of annotated non-coding RNAs. In addition, we have estimated translational efficiencies and identified subsets of poorly-translated genes that may be subject to translational repression. We are currently performing ribosomal profiling of *S. pombe* cells during meiotic differentiation and under stress conditions to investigate how translation is regulated in response to environmental and developmental cues.

341-C High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome

Yaser Hashem[1], *Amedee Des Georges*[1], *Sarah Buss*[6], *Fabrice Jossinet*[3], *Amy Jobe*[2], *Qin Zhang*[5], *Robert Grassucci*[1], *Chandrajit Bajaj*[5], *Eric Westhof*[4], *Susan Madison-Antenucci*[6], *Joachim Frank*[1]

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Ribosomes, the protein factories of living cells, translate genetic information carried by messenger RNAs into proteins, and are thus involved in virtually all aspects of cellular development and maintenance. The few available structures of the eukaryotic ribosome reveal that it is more complex than its prokaryotic counterpart owing mainly to the presence of eukaryote-specific ribosomal proteins and additional ribosomal RNA insertions, called expansion segments. The structures also differ among species, partly in the size and arrangement of these expansion segments. Such differences are extreme in kinetoplastids. Here we present a high-resolution (~5A) cryo-electron microscopy structure of the ribosome of *Trypanosoma brucei* (figure1 below), the parasite that is transmitted by the tsetse fly and that causes African sleeping sickness. The atomic model reveals the unique features of this ribosome (figure2 below), characterized mainly by the presence of unusually large expansion segments and ribosomal protein extensions leading to the formation of four additional inter-subunit bridges. We also find additional rRNA insertions, including one large rRNA domain that is not found in other eukaryotes. Furthermore, the structure reveals the five cleavage sites of the kinetoplastid large ribosomal subunit rRNA chain, which is known to be cleaved uniquely into six pieces, and suggests that the cleavage is important for the maintenance of the *T. brucei* ribosome in the observed structure. We discuss several possible implications of the large rRNA expansion segments for the translation regulation process and we show a possible link between the protein translation initiation process and expansion segments 6 and 7 on the small ribosomal subunit. The structure could serve as a basis for future experiments aimed at understanding the functional importance of these kinetoplastid-specific ribosomal features in protein translation regulation, an essential step towards finding effective and safe kinetoplastid-specific drugs.

Reference:

Hashem *et al*., *Nature* **494**, 385–389 (2013).

Figure 1

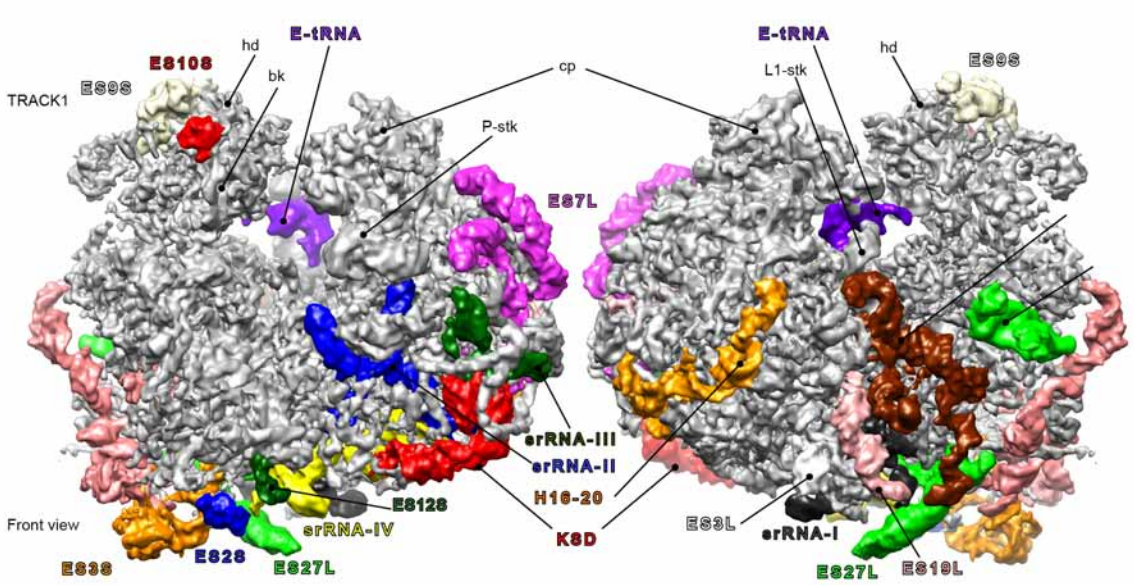
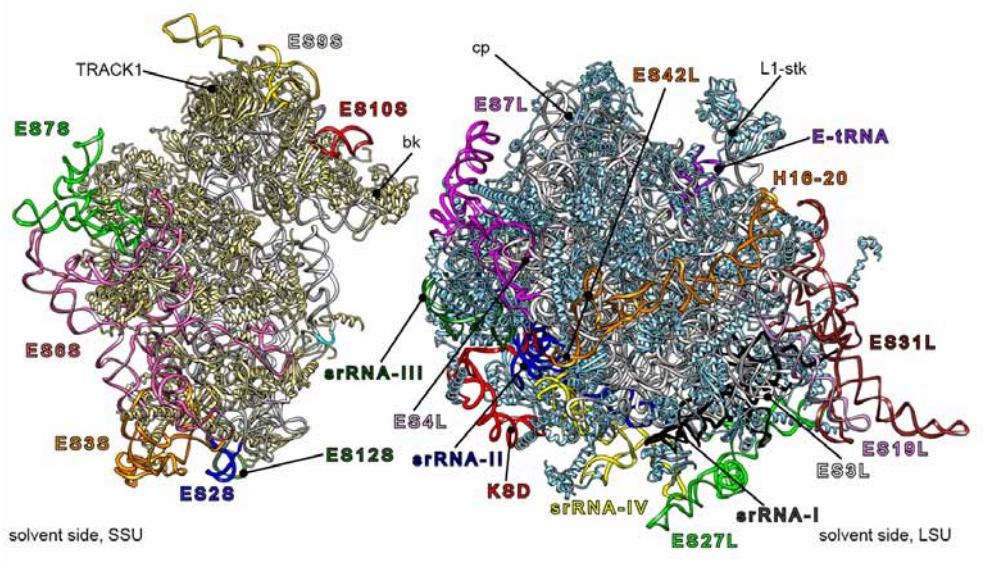


Figure 2



344-C Interactions of modified oligonucleotides with RNA of the prokaryotic and eukaryotic decoding site

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Many known antibiotics that hinder protein synthesis in bacteria, target various functional regions of ribosomal RNA (rRNA). In principle, rRNA function can also be inhibited in a sequence-specific manner by using short oligonucleotides hybridizing with functional rRNA motifs. Indeed, some modified oligonucleotides were shown to hybridize with the RNA of bacterial ribosomes and inhibit the translation process[1]. Typically, the oligonucleotides are designed based solely on the sequence of rRNA, and the secondary and tertiary structures of the targeted rRNA motif are not taken into account. However, to increase the binding affinities of such oligonucleotides their interactions with RNA need to be fully elucidated, taking into account thermodynamic stability, structural and dynamical properties of the RNA target.

We have studied the binding of three S-DNA and 2'O-methyl-RNA decamers complementary to the models of prokaryotic and eukaryotic rRNA decoding sites (A-sites). We have used a structural model of the bacterial ribosomal target: the HX RNA construct[2] (PDB: 3LOA), which contains a fragment of the helix h44 of 16S rRNA.

The thermodynamics of binding was determined by the UV-monitored thermal denaturations and isothermal titration calorimetry (ITC) experiments. We have studied the specificity of the oligonucleotides, by testing the effect of one, two or three mismatches on the binding process. We have also performed 300 ns explicit solvent molecular dynamics (MD) simulations of both rRNA models to characterize the flexibility of the targeted rRNA structures. The fluctuations of the nucleotides indicate which fragments of the target are more susceptible to strand invasion by oligonucleotides. We compare the solution binding experimental studies with the computational predictions derived from the analysis of the MD trajectories. We focus on the relationship between the flexibility of the target fragment and the binding energy between the oligonucleotide and the rRNA.

In addition, we have compared the results obtained for model structures with *in vitro* studies employing whole ribosomal subunits in a cell-free transcription/translation system. We are looking for correlations between the physical parameters of binding of the oligonucleotides with their inhibitory efficiency on bacterial translation. Detailed study of the interaction between modified oligonucleotides and rRNA model structures is crucial for understanding the mechanisms of action of the therapeutic antibacterial oligonucleotide-containing compounds.

[1]Abelian, A, et al. **(2004)**. *Biochem. J.*, 383, Pt 2:201-8.

[2]Dibrov, SM, et al. **(2010)**. *Nucleic Acids Res.*, 38, 13:4458-65.

347-C Rrp5 binds the pre-rRNA at multiple sites and is required for ribosomal processing and assembly

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The large, highly conserved ribosome synthesis factor Rrp5 has 12 S1 RNA binding domains and 7 TRP protein interaction domains, and is required for the early events in both 40S and 60S ribosomal subunit synthesis. In vivo complementation experiments show that the CTD of Rrp5 is required for pre-rRNA cleavage at sites A0-A2 on the pathway of 18S rRNA synthesis, whereas the NTD is required for A3 cleavage on the pathway of 5.8S/25S rRNA synthesis. Using the CRAC UV-crosslinking technique we identified multiple Rrp5 binding sites along the yeast pre-rRNA. The strongest interactions were seen between sequences flanking A2 and the CTD and between sequences flanking A3 and the NTD. The CTD also crosslinked to the U3, U14 and snR10 snoRNAs that are required for normal cleavage at sites A0-A2. In contrast, the NTD crosslinked to the RNA component of RNase MRP, which cleaves site A3. Mathematical modeling of kinetic labeling data and EM analyses both indicate that co-transcriptional cleavage at sites A0-A2 is not stochastic, but occurs during a window when the transcribing polymerase is ~1.5kb 3' to cleavage site A2 and within the 25S rRNA. Notably, a binding site for the Rrp5 CTD, which is required for A0-A2 cleavage, was found in the 25S rRNA region, suggesting that transcription through this Rrp5 binding site in 25S rRNA might set the timing for A2 cleavage. Dramatic support for a specific site in 25S was provided by crosslinking and ligation of hybrids (CLASH), which showed that the Rrp5 binding site in 25S base-pairs to the sequencing flanking cleavage sites A2. We propose that this long-range interaction, facilitated by Rrp5, plays a key role in coordinating the timing of pre-ribosomal assembly and processing.

350-C Evolutionary decline for a nuclear-encoded human mitochondrial aminoacyl-tRNA synthetase

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Mammalian mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are nuclear-encoded and fulfill their activity in mitochondria. Their genes differ from the ones of cytosolic-adressed aaRSs. Many mammalian mt-aaRSs are of bacterial type and share structural domains with homologous bacterial enzymes of the same specificity, such as human mt aspartyl-tRNA synthetase (AspRS). Despite the fact that the mt-AspRS and AspRS from *E.coli* have a common ancestor numerous functional idosyncrasies/discrepancies were reported for the human mitochondrial enzyme.

In order to enlarge the knowledge on mt-AspRS, the present study investigated a serendipitously amplified PCR fragment, which corresponds to an alternative spliced transcript. This isoform lacks the exon 13 coding for a region in the "Bacterial Insertion Domain" (BID). We showed that the alternative transcript was present in all tested tissues; co-existed with the full-length form, possesses a 5'-and 3'-UTRs, a poly-A tail and was bound to polysomes. The corresponding protein was hard to express *in vitro* and was not detectable *in cellulo* or *in vivo*, strongly suggesting a decreased stability. In addition, bioinformatic analysis revealed that the mean acid identities percentages of BID were divergent in opisthokont and protist sequences and distinguished them from the bacterial and viridiplantae ones. This suggests a loss of evolutionary pressure for this domain in non-viridiplantae AspRSs of mitochondrial location.

Altogether, the combination of released selective pressure with the occurrence of alternative splicing suggests that the new isoform serves as evolutionary playground towards a possible reshaping of the BID. This ongoing decline of the BID underlines a relaxation of the mt enzyme.

353-C Non-ribosomal Interaction Partners of Ribosomal Protein S3

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The ribosomal protein S3 (Rps3) is an essential component of 40S ribosomal subunits. Rps3 contains a substantial amount of positively charged amino acids which facilitate its interactions with the negatively charged ribosomal RNA. Like other ribosomal proteins, Rps3 is prone to aggregation, which may be due to interactions of these regions with negatively charged components of the cytoplasm.

S. cerevisiae Rps3 engages in an interaction with the small ankyrin-repeat protein Yar1. Yar1, a 22 kDa protein, is hypothesized to contain two ankyrin-repeats, which are common protein-protein interaction motives. Yar1 is thought to bind and maintain the solubility of free Rps3 in the cytoplasm and deliver it to pre-ribosomes in the nucleus.

Yeast Rps3 shares 66 % identity and 78 % similarity with its human counterpart. We have been able to show using a yeast-2-hybrid analysis that human Rps3 interacts with the yeast ankyrin-repeat protein Yar1. As Rps3 is highly conserved from yeast to humans and ribosomal proteins undergo rapid turnover when not ribosomal bound, we are investigating whether a similar interaction may occur in human cells. Consequently, we are analyzing an ankyrin-repeat protein candidate which interacts with and may stabilize free human Rps3. These data will be presented here.

356-C Investigating the function of the RNA helicase Prp43 and its cofactor Pfa1 in 40S ribosomal subunit synthesis

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Ribosome biogenesis starts in the nucleus with the formation of pre-ribosomal subunits that undergo maturation steps in the nucleolus and in the cytoplasm after export through the nuclear pore complex. Cytoplasmic 40S maturation steps comprise structural changes and processing of the rRNA, but how these events play together in detail remains elusive.

The DEAH box RNA helicase Prp43 and its cofactor, the G-patch protein Pfa1 participate in multiple steps of ribosome biogenesis. Prp43 removes sno-RNAs from pre-ribosomal particles and is involved in rRNA processing events during both 60S and 40S subunit maturation. However, its exact functions at the different stages of ribosome synthesis are not known. Here we investigated the genetic network of Pfa1 and Prp43 with late pre-40S factors and ribosomal proteins in order to understand the 40S specific role of this enzyme.

359-C Going through the motions: Network analysis reveals conserved intramolecular communication pathways within EF-Tu responsible for ribosome dependent GTPase activation and nucleotide exchange

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During the elongation cycle of bacterial protein synthesis elongation factor (EF) Tu delivers aminoacyl(aa)-tRNAs to the ribosome in a GTP-dependent manner. EF-Tu functions as a checkpoint in translation as correct codon/anticodon base pairing is required to send an activating signal over more than 80Å from the decoding centre on the small (30S) ribosomal subunit to the G domain of EF-Tu bound to the 50S large ribosomal subunit. Following dissociation from the ribosome EF-Tu?•GDP is recycled into its GTP-bound form through the interaction with its nucleotide exchange factor EF-Ts. Both functions require the transmission of signals form the respective interaction sites across EF-Tu to facilitate either GTP hydrolysis or nucleotide release. Little is known about the dynamic features governing signal transmission within a highly conserved protein such as EF-Tu and its particular evolutionary constraints.

Molecular dynamics (MD) simulations of EF-Tu and variants with single amino acid substitutions were performed and subsequently interrogated using network analysis methods. Here we report an intramolecular communication network for EF-Tu representing a complex set of long-range signal transmission pathways. Using this network we identify communication pathways essential for efficient GTPase activation on the ribosome as well as EF-Ts-catalyzed nucleotide exchange. We find that single amino acid substitutions in EF-Tu can change the network organization dramatically and result in significantly reduced communication between domains across a set of universally conserved interdomain bridges. To validate our findings in vitro we have performed Michaelis-Menten analyses to study ribosome stimulated GTP hydrolysis of the EF-Tu•GTP binary complex. Aa-tRNA was omitted to isolate this signal pathway from any aa-tRNA contributions. Consistent with our network analysis, these results reveal that single amino acid substitutions in domain II reduce the stimulatory effect of the 70S ribosome by five-fold, while not affecting 50S-dependent stimulation. Furthermore, variants of EF-Tu that disrupt the conserved interdomain bridges reduce the stimulatory effect of the 70S ribosome to a similar extent. Analyses of the EF-Tu•EF-Ts binary complex revealed a different set of communication pathways promoting nucleotide exchange in EF-Tu which were validated using rapid-kinetics techniques to determine nucleotide binding properties of the respective EF-Tu variants.

Translational Regulation

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 362 C – 398 C, 720 C
Location: Main Hallway & Sanada Foyer

362 C	**One messenger RNA and three initiation codons govern the synthesis of two human Glycyl-tRNA synthetases isoforms**
365 C	**Nanos recruits the CCR4-NOT complex to induce degradation of mRNA targets**
368 C	**DDX3 Regulates Rac1 Translation, Modulates Wnt Signaling and Is Required for Cancer Cell Metastasis**
371 C	**Molecular characterization of SMG1-UPFs complexes**
374 C	**When small non-coding RNAs meet the ribosome: Tuning the translational machinery**
377 C	**Splicing factor SRSF3 represses the translation of Programmed Cell Death 4 mRNA by associating with the 5'UTR**
380 C	**Translation of HTT mRNA with expanded CAGrepeats is regulated by the MID1–PP2A proteincomplex**
383 C	**Exploring the role of the GW182 protein, Gawky (Gw) during early Drosophila melanogaster embryogenesis**
386 C	**NALM-6 acute lymphoblastic leukaemia cell line has elevated level of the subunit b of translation initiation factor 3**
389 C	**Gene silencing using artificial small RNAs derived from a natural RNA scaffold in Escherichia coli**
392 C	**Two Retinoblastoma associated SNVs in RB1 form a RiboSNitch.**
395 C	**Dissecting the regulation of vFLIP expression, a Kaposi's Sarcoma-associated Herpesvirus tumorigenesis factor**
398 C	**Unravelling the role of dimerization for the STAR-domain RBP, GLD-1**
720 C	**Equilibrium-dependent ribosomal recoding mechanisms in RNA viruses**

362-C One messenger RNA and three initiation codons govern the synthesis of two human Glycyl-tRNA synthetases isoforms

Jana Alexandrova[1], Caroline Paulus[1], Joëlle Rudinger-Thirion[1], Magali Frugier[1]

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Human Glycyl-tRNA synthetase (GlyRS) is a housekeeping enzyme with a key role in protein synthesis, responsible for the charging of glycine on its cognate tRNA. In metazoans, there is a unique gene, GARS, which encodes both cytoplasmic and mitochondrial GlyRSs. At least 3 putative initiator AUG codons were found amongst the 300 first nucleotides of the mRNA: AUG#1 is not in frame and would initiate the translation of a 32 amino-acids peptide, AUG#2 and AUG#3 initiates the translation of the mitochondrial GlyRS precursor and the cytosolic form of GlyRS, respectively.

AUG codons were mutated, alone or in combination, and the expression efficiency as well as the subcellular localization (mitochondria, cytosol) of each GlyRS variant were determined. Our results show that, this mRNA codes mainly for the cytosolic form of GlyRS. This expression becomes mainly mitochondrial when AUG#1 is removed and exclusively mitochondrial when AUG#1 and AUG#3 are mutated together. On the contrary, the simultaneous presence of AUG#1 and AUG#2 does not allow any GlyRS translation. *In vitro* translation assays not only confirmed these results but also showed that AUG#1 is an efficient initiator codon (the design of a frame-shift mutant leads to the synthesis of an extra-long GlyRS).

Based on these observations, we hypothesized that the ribosome initiates translation at AUG#1, translates the 32 amino acid long peptide through AUG#2, terminates at the stop codon and reinitiates translation at AUG#3 to produce the cytosolic GlyRS. However, when mitochondrial GlyRS is needed, the ribosome reinitiate translation at AUG#2, suggesting that the peptide translation is prematurely terminated. Indeed, the introduction of a stop codon between both initiation sites (AUG#1 and AUG#2) allowed recovering the synthesis of the mitochondrial form of GlyRS.

The very short 5' UTR present in front of AUG#1 (only 15 nucleotides) as well as a dense structure characterized by a high GC content suggest the presence of a particular initiation mechanism. Moreover, the sequence of the short peptide (rich in Arginine and Proline residues) supports a regulatory mechanism based on the cellular level of Arg and Pro, which metabolism depends on mitochondria.

365-C Nanos recruits the CCR4-NOT complex to induce degradation of mRNA targets

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The Nanos protein is a non-specific RNA-binding protein, which together with Pumilio represses translation of the maternally deposited *hunchback* (*hb*) mRNA and plays a major role in the establishment of the anterior-posterior body axis in *Drosophila melanogaster*. Previous studies have indicated that the *hb* 3' UTR is sufficient for translational repression and that reporter mRNAs containing this 3' UTR are translationally repressed and deadenylated in a Nanos- and Pumilio-dependent manner. However, the exact molecular mechanism of deadenylase complex recruitment to the mRNA reporter has so far remained elusive. Here we investigated the role of Nanos in the regulation of the *hb* 3' UTR. The Nanos protein contains an N-terminal region predicted to be unstructured and two conserved C-terminal CCHC type zinc finger motifs. Using coimmunoprecipitation assays in *Drosophila* S2 cells, we show that Nanos interacts with both the CCR4-NOT and PAN2-PAN3 deadenylase complexes. Furthermore, we mapped the deadenylase-binding region to a fragment within the unstructured part of the protein. An *in vitro* pull-down experiment revealed that Nanos interacts directly with the NOT module of the CCR4-NOT complex. We also show that the deadenylase-binding region of Nanos is required to elicit mRNA degradation via the 5'-to-3' decay pathway. In addition, our results show that tethered Nanos can promote both translational repression and mRNA degradation independently of Pumilio. Interestingly, the three human Nanos proteins, Nanos 1-3, also interact with the CCR4-NOT deadenylase complexes in human cells. Similar to *Dm* Nanos, human Nanos 1-3 promote the degradation of bound mRNAs. We conclude that Nanos proteins can recruit the CCR4-NOT complex to mRNA targets independently of Pumilio and that this activity is conserved amongst Nanos orthologs.

368-C DDX3 Regulates Rac1 Translation, Modulates Wnt Signaling and Is Required for Cancer Cell Metastasis

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DDX3 is a DEAD-box RNA helicase involved in multiple steps of gene expression. DDX3 is upregulated in hepatocellular carcinoma tissues and in aggressive breast epithelial cancer cell lines, and is considered as a biomarker of metastasis in squamous cell/ adenosquamous carcinomas. The genetic and functional interactions between DDX3 and ß-catenin have been observed in the Wnt type of medulloblastoma. Therefore, DDX3 is a potential oncogene. To reveal oncogenic roles of DDX3 in metastasis, we analyzed phenotypes caused by DDX3 depletion in HEK293, cervical cancer HeLa and neuroblastoma N2A cell lines. In these cell lines, DDX3 depletion increases cell-cell adhesion and decreases cell-extracellular matrix adhesion, cell migration and invasion. Metastasis assays further demonstrate that DDX3 is required for efficient metastasis of cancer cells. These results suggest that DDX3 may play an oncogenic role in controlling cancer cell adhesion, migration and invasion/metastasis via modulating cytoskeleton organization. To uncover potential targets of DDX3 involved in cytoskeleton remodeling, we performed pathway analysis of DDX3 targets that are regulated at the level of mRNA translation, and identified multiple pathways involved Rac1 functions. Our following experiments demonstrate that DDX3 may activate Rac1 translation perhaps by resolving its 5'UTR secondary structure. Moreover, we performed qRT-PCR array analysis of cell motility genes and found that transactivation of the Wnt/ß-catenin target genes was down-regulated in DDX3 knockdown cells. Our results further showed that DDX3 depletion decreases ß-catenin stability and attenuates Wnt/ß-catenin signaling, and that Rac1 can rescue ß-catenin expression and cell adhesion in DDX3 knockdown cells. Rac1 is a major factor of the Wnt PCP pathway that controls cell movement and also regulates the stability of ß-catenin in the canonical Wnt pathway. Thus, our study unveils a molecular mechanism by which DDX3 controls cancer metastasis via regulating Rac1 and ß-catenin signaling of cancer cells and supports DDX3 as a culprit in Wnt type tumors.

371-C Molecular characterization of SMG1-UPFs complexes

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Non-sense Mediated mRNA Decay (NMD) allows the recognition of Premature Termination Codon (PTC)-containing mRNAs[1]. This quality control mechanism is crucial to avoid putatively toxic truncated proteins in the cell[2]. PTCs are recognised by the SURF complex consisting of eRF1/eRF3/Upf1/SMG1-8-9 [3, 4]. Subsequent interaction of the SURF complex with the Upf2-3-Exon Junction Complex (EJC) triggers SMG1-mediated Upf1 phosphorylation[3,5]. This phosphorylation is the key event initiating the mRNA decay cascade [1,5].

In order to dissect the molecular details of activation of the NMD pathway *in vitro*, we generated SMG1 PIK-like kinase alone, SMG1-9 and SMG1-8-9 complexes. The SMG1 kinase alone or in complex with SMG9 or SMG8-9 is able to phosphorylate UPF1 expressed in insect cells as a non-phosphorylated form. We determined structures of SMG1-8-9 and a SMG1-8-9-UPF complex at low resolution by single particle electron microscopy. We solved the structures of all three UPF2 MIF4G domains and show that the MIF4G-3 domain not only binds UPF3 but also SMG1 in a non-competitive manner [6]. In fact, this could be the key interaction between the SURF and the UPF2-3-EJC complexes triggering UPF1 phosphorylation, translation termination at the PTC and faulty mRNA degradation.

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374-C When small non-coding RNAs meet the ribosome: Tuning the translational machinery

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The functions of ribosomes in translation are complex and involve different types of activities critical for decoding the genetic code, linkage of amino acids via amide bonds to form polypeptide chains, as well as the release and proper targeting of the synthesized protein. Non-protein-coding RNAs (ncRNAs) have been recognized to be crucial in establishing regulatory networks.[1] However all of the recently discovered ncRNAs involved in translation regulation target the mRNA rather than the ribosome. The main goal of this project is to identify potential novel ncRNAs that directly bind and possibly regulate the ribosome during protein biosynthesis. To address this question we applied various stress conditions to the archaeal model organism *Haloferax volcanii* and deep-sequenced the ribosome-associated small ncRNA interactome. In total we identified 6.250 ncRNA candidates. Significantly, we observed the emersed presence of tRNA-derived fragments (tRFs). These tRFs have been identified in all domains of life and represent a growing, yet functionally poorly understood, class of ncRNAs. Here we present evidence that tRFs from *H. volcanii* directly bind to ribosomes. In the presented genomic screen of the ribosome-associated RNome a 26 residue long fragment originating from the 5' part of valine tRNA was by far the most abundant tRF. The Val-tRF is processed in a stress- dependent manner and was found to primarily target the small ribosomal subunit *in vitro* and *in vivo*. As a consequence of ribosome binding, Val-tRF reduces protein synthesis by interfering with peptidyl transferase activity. Therefore this tRF functions as ribosome-bound small ncRNA capable of regulating gene expression in *H. volcanii* under environmental stress conditions probably by fine-tuning the rate of protein production.[2] Currently we are investigating the binding site of this tRF on the 30S subunit in more detail.

[1] Mattick J. and Makunin I.V. (2006) Hum. Mol. Genetcis, 15:R17-R29

[2] Gebetsberger J. et. al. (2012) Archaea, Article ID 260909

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

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SRSF3, an SR family of RNA binding proteins, is known to regulate alternative splicing of pre-mRNA in the nucleus and facilitate export of spliced mRNA to the cytoplasm. Despite its well established roles in mRNA processing in the nucleus, the mechanism by which it regulates the fate of exported mRNA in the cytoplasm remains poorly understood. Here, we provide evidence that SRSF3 not only regulates alternative splicing of the programmed cell death 4 (PDCD4) gene in the nucleus, but also modulates the translation of the PDCD4 mRNA in the cytoplasm. We showed that SRSF3 knockdown specifically enhanced PDCD4 translation as indicated by increased mRNA in polysome fractions whereas SRSF3 overexpression showed the opposite effect, suggesting that SRSF3 is involved in repressing PDCD4 translation. Moreover, we found that SRSF3 and PDCD4 mRNA colocalized in P-bodies (PBs) where translationally silenced mRNAs are deposited and the localization of PDCD4 mRNA in PBs was abrogated in SRSF3 knockdown cells. Ribo-IP experiments established that SRSF3 binds to PDCD4 mRNA, preferentially via its 5'UTR region, to mediate translational repression. Together, these data suggest that SRSF3 may function as a oncoprotein in mammalian cells, at least in part, through repressing the translation of a critical cell death regulator.

380-C Translation of HTT mRNA with expanded CAGrepeats is regulated by the MID1–PP2A proteincomplex

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Expansion of CAG repeats is a common feature of various neurodegenerative disorders, including Huntington's disease. Here we show that expanded CAG repeats bind to a translation regulatory protein complex containing MID1, protein phosphatase 2A and 40S ribosomal S6 kinase. Binding of the MID1–protein phosphatase 2A protein complex increases with CAG repeat size and stimulates translation of the CAG repeat expansion containing messenger RNA in a MID1-, protein phosphatase 2A- and mammalian target of rapamycindependent manner. Our data indicate that pathological CAG repeat expansions upregulate protein translation leading to an overproduction of aberrant protein and suggest that the MID1-complex may serve as a therapeutic target for the treatment of CAG repeat expansion disorders.

383-C Exploring the role of the GW182 protein, Gawky (Gw) during early Drosophila melanogaster embryogenesis

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The GW182 protein family is composed of multiple members, each having a high percentage of glycine-tryptophan (GW) and troptophan-glycine (WG) repetitive amino acid sequences and an RNA recognition motif on the C-terminal end. The *Drosophila* GW182 single homologue, *Gawky* (*Gw*), has been shown to participate in the microRNA (miRNA) repression pathway. A mutation in the *gw* gene results in an abnormal number of centrosome, chromosome mis-segregation and disrupted microtubule network in the nuclear mitosis during early embryogenesis (0-2hrs after egg deposition). Our work shows that Gw interacts with centrosomal structural protein centrosomin (CNN) and the interaction is mediated by RNA. Centrosomes have been seen adjacent to endogenous Gw bodies and the adjacent pattern is mitosis-phase specific in the early embryo. A subset of mRNA related to centrosomal organization function were found to associate with Gw. Notably, conserved miRNA-277-binding sites were predicted to be in the 3' untranslated regions of 4 transcripts responsible for centrosomal organization that also interact with Gw. These results suggested that Gw potentially plays an vital role of organizing centrosome duplication.

386-C NALM-6 acute lymphoblastic leukaemia cell line has elevated level of the subunit b of translation initiation factor 3

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About 75 cases of acute lymphoblastic leukemia (ALL) are diagnosed yearly in the Czech Republic and many more throughout the world. The majority of these cases are in children and young adults, making ALL the most common form of malignancy in these age groups. The treatment protocols of ALL are complex and use 6–12 drugs. Many of those drugs directly or indirectly target translation, for example mTOR pathway. We studied possible role of translation initiation factor 3 (eIF3) in ALL pathogenesis. eIF3 is a multi-subunit complex participating in all steps of translation initiation. Some of its 13 subunits are also connected to fundamental cell processes such as protein degradation, cell cycle control, differentiation and apoptosis. Down-regulated or up-regulated expression of some of the eIF3 subunits was found to be typical for a variety of human cancers.

We quantified expression of individual eIF3 subunits (a, b, d, e, f, g, h, i, and j) by Real-time RT-PCR in lymphoblastic cell lines NALM-6, RS4;11, REH, TOM-1 carrying chromosomal aberrations characteristic for main types of ALL. As a non-leukemic control, we employed NC-NC cell line that represents B-cell precursor immortalized by EBV. Statistical evaluation of results revealed that the expression of eIF3b is significantly higher in NALM-6 than in REH, RS4;11, and TOM-1 cell lines. The level of eIF3b mRNA is comparable between NC-NC and NALM-6. The up-regulation of eIF3b in NALM-6 was also confirmed by quantitative Western blot analyses. Next, we tested an effect of eIF3b on growth speed of the ALL cell lines and found strong correlation between eIF3b expression level and their doubling time. NALM-6 and NC-NC grew faster and better than the other cell lines. On the contrary, TOM-1 grew the most slowly even in the presence of 20% FBS.

However, eIF3b subunit has not been considered to be the subunit of eIF3 which affects general translation primarily; we assume that eIF3b level could be somehow connected to the rate of translation. Our preliminary experiments also suggest that eIF3b expression level reflects rather a particular stage of B-cell development, which the particular ALL cell line is derived from, than a direct or indirect influence of specific chromosomal gene fusion.

Regarding NALM-6, the corresponding leukaemia is very aggressive, refractory to treatment, and patients have very poor chances of recovery. We presume that deregulation of translation can contribute to poor prognosis of those patients.

389-C Gene silencing using artificial small RNAs derived from a natural RNA scaffold in Escherichia coli

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Small noncoding RNAs (sRNAs) function as central regulators in *E. coli* in response to diverse environmental growth conditions. While gene silencing by sRNAs is known to occur through RNA-RNA interactions, the detailed mechanisms underlying base-pairing between sRNA and target mRNAs in the cell remain unclear at present. In the current study, the mechanism of base pairing *in vivo* was explored using artificial sRNA (afsRNA) loaded with various target recognition sequences. An artificial small RNA (afsRNA) scaffold was designed from a natural sRNA, SibC. Using the *lacZ* reporter system, the gene silencing effects of afsRNAs were examined in *E. coli*. Substitution of the original target recognition sequence with a new sequence recognizing *lacZ* mRNA led to effective reduction of *lacZ* gene expression. The target recognition sequence was shortened to 10 nt without significant loss of gene silencing, although this minimal length was limited to specific target mRNA sequences. The presence of mismatched or unmatched regions in the middle of the target recognition sequences of afsRNAs hindered gene silencing, but internal loop-forming afsRNAs were more effective in gene silencing than bulge-forming afsRNAs. Notably, gene silencing by afsRNA was not decreased, but increased upon *hfq* disruption in *E. coli*, particularly when interactions between afsRNA and mRNA were weak, suggesting that Hfq is possibly involved in destabilization of the RNA-RNA duplex. This Hfq function, opposite to its known role in enhancing gene silencing, may contribute to reducing the off-target effects of sRNA caused by unwanted target recognition through short base-parings, thereby increasing target specificity.

392-C Two Retinoblastoma associated SNVs in RB1 form a RiboSNitch.

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Retinoblastoma (RB1) is a negative regulator of the cell cycle and also involved in tumor suppression. Recent computational analysis of known, disease-associated Single Nucleotide Variants (SNVs) in the human genome suggest that two disease-associated SNVs found in the 5' UTR of the RB1 gene alter the mRNA transcript structure. These two mutations, G17C and G18U in RB1's 5' UTR, are also predicted to affect the structure of a putative Internal Ribosome Entry Site (IRES). Using Selective 2'-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) we have analyzed both the wild type (WT) RB1 and the RB1 mutants G17C and G18U. The data reveal significant changes in SHAPE reactivity in both mutants compared to the WT along with structural changes to the IRES site, consistent with the computational prediction. The RB1 5' UTR is thus a RiboSNitch, and our data suggest that SNV induced conformational change in mRNA is likely a drive of oncogenesis.

395-C Dissecting the regulation of vFLIP expression, a Kaposi's Sarcoma-associated Herpesvirus tumorigenesis factor

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Kaposi's Sarcoma-associated Herpesvirus (KSHV) is an oncogenic virus, the etiological agent of Kaposi's Sarcoma (KS); it is also associated with multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL). The infection is mainly latent in KSHV-induced tumour cells with only a few viral genes expressed that contribute to tumorigenesis. Among these, vFLIP interacts with the NFkB pathway to trigger the expression of anti-apoptotic and pro-inflammatory genes.

The expression of vFLIP is mediated by an unusual internal ribosomal entry site (IRES) element. Using an *in vitro* system to dissect vFLIP IRES function we identified that a minimal IRES domain is located within a coding region upstream of the vFLIP gene. Furthermore, using specific inhibitors and proteomic analysis, we found that eIF4A and eIF4G, but also eIF4E, are required for initiation of translation on the vFLIP IRES. Novel IRES-interacting proteins, such as Y box-binding protein 1, were also identified. Currently, we are investigating the requirement of the IRES for these factors and analysing the RNA structure of the vFLIP IRES. By dissecting the structure and function of the vFLIP IRES we will determine how vFLIP expression is regulated during latent KSHV infection. This will deepen our understanding of the viral trigger responsible for NFkB activation and the oncogenic properties of KSHV. The ultimate aim is to define new mechanisms to inhibit KSHV-induced tumour formation.

398-C Unravelling the role of dimerization for the STAR-domain RBP, GLD-1

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RNA-binding proteins (RBPs) are critical regulators of gene expression. Indeed, whether a particular mRNA is translated, repressed, or degraded, depends on its RBP and regulatory RNA interactions. The STAR family of RBPs includes developmental regulators such as C. elegans GLD-1, which is a key regulator of germ cell development with loss of GLD-1 resulting in a germline tumour. GLD-1 binds approximately 10% of all germline transcripts via GLD-1 binding motifs (GBMs) in UTRs[1] resulting in translational repression and/or stabilisation of its target mRNAs[2]. Dimerization of RBPs is generally thought to be required for their functions in RNA-recognition, mRNP complex formation, RNA oligomerization and controlling the balance between an RBP and its targets. GLD-1 and its closest STAR protein family homologs, e.g. Quaking (QKI), can homodimerize[3,4]. Inhibiting QKI dimerization causes embryonic lethality although the molecular basis for this is unknown[5]. We find that dimerization of GLD-1 is generally not required for its function as it does not phenocopy the null mutant and the global RNA binding ability of dimerization mutant GLD-1 is largely unchanged. However, inhibiting GLD-1 dimerization does leads to a high percentage of sterile worms. We hypothesize that GLD-1 dimerization is involved in regulating specific mRNAs, possibly affecting different aspects of mRNA regulation for different targets.

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720-C Equilibrium-dependent ribosomal recoding mechanisms in RNA viruses

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RNA viruses have evolved mRNA-mediated recoding mechanisms to regulate gene expression at the translational level. Two of such mechanisms, codon readthrough and programed -1 frameshift, allow the ribosome to bypass a stop codon and synthesize fusion viral proteins. The frequency by which these events occur is important for efficient viral infectivity, and is regulated by domains in the translating mRNA (in the case of the SARS-corona virus and the murine leukemia virus, the domains are pseudoknots). Although aspects of frameshifting have received considerable attention, an understanding of the RNA structures and structural rearrangements that influence the efficiency is lacking. Our results from murine leukemia virus indicate that retroviral gene expression is regulated by a dynamic, proton-driven equilibrium between an active, read-through permissive, and an inactive pseudoknot conformation. We are now working towards a complete structural and mechanistic understanding of the frameshifting frequency in SARS-corona virus by combining structural studies with biochemical in-vivo and in-vitro experiments. Specifically, to understand the basis for how the recoding frequency is maintained, we determined the structures of the mRNA signal in both frameshifting conformations: permissive and non-permissive. These structures allowed us to engineer mutants to test our equilibrium model and generalize the results for equilibrium-dependent recoding to include both readthrough and frameshift mechanisms.

3' end processing

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 401 C – 413 C
Location: Main Hallway & Sanada Foyer

401 C	**Post-transcriptional regulation of COX-2**
404 C	**Transcriptional and translational profiles in stimulated T lymphocytes**
407 C	**ENHANCER OF RNA INTERFERENCE -1-LIKE-1: ONE MORE PLAYER IN THE RNA PROCESSING GAME OF THE CHLOROPLAST**
410 C	**The U7 snRNP revisited: a complex of the core U7 snRNP, FLASH and multiple polyadenylation factors controls 3' end processing of histone pre-mRNAs in vertebrates and invertebrates**
413 C	**A triple helix structure is able to functionally replace a poly(A) tail**

401-C Post-transcriptional regulation of COX-2

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The oxidative conversion of arachidonic acid to prostaglandin H_2 is carried out by a set of two enzymes termed cycloxygenases, abbreviated as COX. COX-1 is constitutively expressed in normal tissues, while COX-2, which is not expressed in normal tissues, is transiently induced from external stimuli, such as pro-inflammatory cytokines. COX-2 is also overexpressed in numerous cancers. We show that COX-2 protein expression is constitutive in a lung cancer cell line, A549, but not expressed in a normal bronchial cell line, Beas2B. Previous work from our lab has shown that COX-2 has two polyadenylation signals present in its 3'UTR that can potentially be utilized. Alternative polyadenylation is a post-transcriptional mechanism by which mRNAs can produce variable 3' untranslated region (UTR) lengths is through usage of alternative poly(A) sites. Our RNAse H-coupled RT-PCR data indicates that both COX-2 mRNA isoforms, resulting from usage of two different poly(A) sites, are transcribed in A549 lung cancer cells. Another means of post-transcriptional regulation is mediated through microRNA repression. We have Real-Time qPCR data and microarray data that show decreased expression of specific miRs in lung cancer cells as compared to normal lung cells. The biological function of COX-2 is to produce prostaglandins; we have also demonstrated that COX-2 specific miRs can modulate resulting prostaglandins produced. We speculate that many post-transcriptional mechanisms work in concert to regulate COX-2 expression, which may explain the employment of an alternative poly(A) signal.

404-C Transcriptional and translational profiles in stimulated T lymphocytes

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The many processing steps to which RNA polymerase II transcripts are subjected include cleavage and polyadenylation within the 3' untranslated region. Many genes have multiple polyadenylation sites, whose differential use results in different mRNA isoforms. Although the processing efficiency for pre-mRNAs is largely determined by the strength of their polyadenylation signals, recent studies showed that polyadenylation site choice can be influenced by several protein factors that have long been known to be involved in pre-mRNA processing. One of the model systems in which systematic changes in the use of polyadenylation sites are studied is T cell activation, but similar changes have also been observed in development and cell differentiation.

In this study we undertook a systematic investigation of the pattern of poly(A) site selection and its influence on the level of protein synthesis during activation of T cells. By deep sequencing of mRNA 3' ends we mapped polyadenylation sites in resting and activated murine and human lymphocytes. At the same time we measured global transcript and protein levels. Through computational analysis we inferred genes that undergo a significant shift in polyadenylation site use between the two states and we characterized the properties of the respective sites. Because shorter transcripts isoforms lack sequence elements with negative impact on mRNA stability and translation -such as miRNA binding sites- it has been hypothesized that the shorter transcript isoforms are associated with an increased protein production as found in highly proliferating cancer cells. Here, we investigated this hypothesis by measurements of mRNA, 3' end processing and protein levels in primary cells under noncancerous conditions.

407-C ENHANCER OF RNA INTERFERENCE -1-LIKE-1: ONE MORE PLAYER IN THE RNA PROCESSING GAME OF THE CHLOROPLAST

Glykeria Mermigka[1], Ioannis Vlatakis[1], Eugenia vamvaka[2], Jutta Helm[2], Heiko Schumacher[2], Kriton Kalantidis[3]

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

410-C The U7 snRNP revisited: a complex of the core U7 snRNP, FLASH and multiple polyadenylation factors controls 3’ end processing of histone pre-mRNAs in vertebrates and invertebrates

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Animal replication-dependent histone pre-mRNAs are processed at the 3' end by endonucleolytic cleavage that is not followed by polyadenylation. The cleavage reaction is catalyzed by CPSF73 and depends on binding of the U7 snRNP to a sequence in histone pre-mRNA downstream of the cleavage site. In the past 10 years the U7 snRNP has been portrayed as a simple complex of an approximately 60-nucleotide U7 snRNA and a unique Sm ring in which the spliceosomal-specific proteins SmD1 and SmD2 are replaced by the related Lsm10 and Lsm11. We recently showed that in both vertebrates and invertebrates, Lsm11 interacts with a protein called FLASH and that this interaction is essential for processing. We now demonstrate that the U7 snRNP isolated from mammalian and *Drosophila* nuclear extracts is stably associated with FLASH and a number of polyadenylation factors that we refer to as the Histone pre-mRNA Cleavage Complex (HCC). The association of the HCC with the core U7 snRNP critically depends on the interaction between Lsm11 and FLASH. The mammalian HCC consists of symplekin, CstF64 and all six CPSF subunits, including the CPSF73 endonuclease. FLASH is severely limiting in mammalian nuclear extracts, likely explaining the inability of previous studies to identify the composite structure of the U7 snRNP and its association with the polyadenylation factors. In *Drosophila* nuclear extracts, FLASH is abundant and quantitatively associated with the U7 snRNP. The *Drosophila* HCC has a similar composition to the mammalian HCC but lacks two CPSF subunits, Fip1 and CPSF30. In both mammalian and *Drosophila* nuclear extracts, the composite U7 snRNP bearing FLASH and multiple polyadenylation factors is subsequently recruited to histone pre-mRNA for 3' end processing. However, of these polyadenylation factors only three are essential for the cleavage reaction *in vivo*: CPSF73, CPSF100 and symplekin. The other subunits are likely passive spectators or play nonessential regulatory roles. Collectively, our studies revealed an unexpected complexity of the U7 snRNP and suggest that this factor functions as an RNA-guided multi-subunit nuclease where the U7 snRNA recognizes the substrate and together with three proteins of the Sm ring, SmD3, SmB and Lsm10, defines the site of cleavage that is carried out by the catalytic component, CPSF73.

413-C A triple helix structure is able to functionally replace a poly(A) tail

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The MALAT1 locus is commonly misregulated in many human cancers and produces an abundant long nuclear-retained noncoding RNA. Despite being transcribed by RNA polymerase II, the 3' end of MALAT1 is produced not by canonical cleavage/polyadenylation but instead by recognition and cleavage of a tRNA-like structure by RNase P. Mature MALAT1 thus lacks a poly(A) tail yet is expressed at a level higher than many protein-coding genes in vivo. We find that the 3' ends of MALAT1 and the MEN beta long noncoding RNAs are protected from 3'-5' exonucleases by highly conserved triple helical structures. Surprisingly, when these structures are placed downstream from an ORF, the transcript is efficiently translated in mammalian cells despite the lack of a poly(A) tail. The triple helix therefore also functions as a translational enhancer, and mutations in this region separate this translation activity from simple effects on RNA stability or transport. This unusual form of translational control appears to be highly evolutionarily conserved as we now find that reporter mRNAs ending in a triple helix are efficiently translated in yeast. We are additionally investigating if endogenous MALAT1 may actually produce short peptides. These results provide new insights into how transcripts that lack poly(A) tails are stabilized and regulated and suggest that RNA triple helical structures likely have key regulatory functions in vivo.

RNA Turnover

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 416 C – 446 C
Location: Main Hallway & Sanada Foyer

416 C	**Exploring the role of mouse DEAH helicase, skiv2l2, in processing and degradation of non coding RNAs in neuronal N2A cells using high throughput pA-seq analysis**
419 C	**THE NOT2/NOT5 MODULE OF THE CCR4-NOT COMPLEX IS REQUIRED FOR ASSEMBLY OF THE EXOSOME**
422 C	**The structural and functional organization of the TRAMP complex**
425 C	**Retroviral strategies for NMD evasion**
428 C	**Identification of novel UPF1 target transcripts by direct determination of whole transcriptome stability.**
431 C	**Expression of nonsense-mediated decay factors is controlled by conserved feedback loops and responds to salt stress in plants**
434 C	**SMG7 recruits the CCR4-NOT complex for degradation of NMD targets**
437 C	**Global analysis of exosome target introns**
440 C	**Structural insights into the Dhh1-Pat1 interaction**
443 C	**MKT1: a hub in a post-transcriptional regulatory network**
446 C	**Enriched density of UPF1 in 3' untranslated regions results from its translation-dependent displacement from coding sequences**

416-C Exploring the role of mouse DEAH helicase, skiv2l2, in processing and degradation of non coding RNAs in neuronal N2A cells using high throughput pA-seq analysis

James Anderson[1], Jane Dorweiler[1], Fengchao Wang[1], Jun Zhu[2], Stephen Munroe[1]

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Production and function of RNAs demands accurate transcription, processing and protein binding that is interogated by a number of mechansims. RNA surveillance and degradation insures that inaccurately transcribed or processed RNAs are efficiently removed by nuclease degradation. The RNA surveillance complex, TRAMP, has been characterized and studied quite effectively in the yeast model organism, Saccharomyces cerevisiae. Proteins orthologous to yeast TRAMP complex proteins were identified in mus musculus, however the study of RNA surveillance in mammals has only recently begun. In this work we have used small interfering RNAs to target orthologs of yeast proteins that function in RNA surveillance (Mtr4p and Rrp6p) in mouse N2A cells.

We used paired-end high-throughput sequencing of polyadenylated RNAs (pA-seq) to quantify the effects of *mMtr4* and *mRrp6* knockdowns on RNA surveillance and processing. We demonstrate that there is no difference in the accumulation or location of polyadenylation in protein coding mRNAs upon depletion of mMtr4 or mRrp6. Several targets of TRAMP mediated RNA surveillance from work in yeast do accumulate as polyadenylated RNAs in the *mMtr4*KD compared to control knockdowns. We also identified a novel target of *mMtr4* dependent RNA surveillance. The 5' leading portion of a pri-miRNA is liberated from the pre-miRNA by Drosha cleavage, and generally thought to be degraded. Our data demonstrate that *mMtr4* plays an important role in degrading these byproducts of miRNA processing. We continue to mine the current dataset for familiar, new or novel targets of mMtr4 and will report our findings.

419-C THE NOT2/NOT5 MODULE OF THE CCR4-NOT COMPLEX IS REQUIRED FOR ASSEMBLY OF THE EXOSOME

Olesya Bukach[1], Virginie Ribaud[1], Geoffroy Colau[1], Martine Collart[1]

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The exosome is a conserved eukaryotic multi-subunit complex that plays a key role in processing and/or degradation of essentially all types of cellular RNAs. A different conserved multi-subunit complex known to specifically contribute to degradation of mRNAs is the Ccr4-Not complex. It carries Ccr4, the major cellular deadenylase in the yeast *S. cerevisiae,* several Ccr4-associated factors (Caf1, Caf40 and Caf130) and 5 Not proteins (Not1-5). In this study, we determine that one functional module of the Ccr4-Not complex composed of Not5 and Not2 contributes to the functional assembly of the exosome. Indeed, both Not2 and Not5 are important for exosome-dependent processing of 7S rRNA *in vivo*, and their deletion suppresses improper processing of the 5.8 S + 30 rRNA due to lack of the Rrp6 exonculease. The N-terminal domain of Rrp4 interacts with Rrp44 in the 10-subunit exosome associated with RNA and is improperly integrated into exosome complexes in the absence of Not2 or Not5. These results identify for the first time cellular components important for exosome assembly *in vivo*.

422-C The structural and functional organization of the TRAMP complex

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The TRAMP complex is involved in the processing and surveillance of various non-coding RNAs produced by all three RNA polymerases in the cell nucleus. TRAMP adds an oligo-adenylated tail to the 3' end of selected RNAs and prompts their degradation by the exosome. The polyadenylation core of the TRAMP complex consists of the non-canonical poly(A)polymerase Trf4 and the zinc-knuckle protein Air2 (or their paralogues Trf5 and Air1). The delivery of polyadenylated RNAs to the exosome is likely mediated by the third subunit of the TRAMP complex, the RNA helicase Mtr4.

The RNA targets of TRAMP have been found to carry short oligoadenylated tails *in vivo*. It has been suggested that Mtr4 restricts the number of adenosines added by Trf4-Air2 by recognizing the 3' end of the RNA that is being polyadenylated. To visualize how the interaction between Mtr4 and Trf4-Air2 might underpin their regulation, we have studied the overall architecture of the *S. cerevisiae* TRAMP complex by combining SAXS reconstructions and crystal structures with interaction data obtained by crosslinking, mass spectrometry, biochemical and biophysical experiments. The results challenge a direct read-out mechanism of the poly-adenylated 3' end by Mtr4 and instead suggest a model where RNAs are first unwound by the Mtr4 helicase and then fed to the poly(A)polymerase.

425-C Retroviral strategies for NMD evasion

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The nonsense-mediated mRNA decay pathway recognizes and degrades transcripts containing long 3' untranslated regions (3'UTRs). To counteract this activity and maintain proper gene expression, retroviruses have evolved RNA elements capable of protecting transcripts from 3'UTR length-dependent decay. The best-characterized retroviral NMD-antagonizing element, the RNA stability element (RSE) of the Rous Sarcoma Virus, stabilizes both retroviral and synthetic reporter mRNAs containing long 3'UTRs. This large (~ 400 nt) RNA segment sits immediately downstream of the viral *gag* termination codon, preventing it from being recognized as premature. Using an RNA-based affinity purification approach, we have identified a complex of proteins specifically recruited to the RSE. These proteins are currently under investigation for potential roles in stabilizing retroviral and cellular RNAs.

Second, we find that retroviral recoding elements that promote translational frameshifting or readthrough antagonize NMD at two distinct steps. Relatively frequent stop codon bypass can reduce steady-state accumulation of Upf1 in mRNPs, disrupting its ability to monitor 3'UTR length. In addition, using variants of the Moloney murine leukemia virus (M-MLV) recoding pseudoknot, we find that less frequent readthrough events permit recovery of Upf1 binding to mRNPs but remain able to inhibit degradation of mRNAs containing long 3'UTRs. Our data indicate that diverse viral and cellular recoding sequences can similarly inhibit NMD, suggesting that suppression of NMD is a general feature of translational readthrough. We are currently investigating the ability of translational readthrough to disrupt the molecular events preceding commitment to nonsense-mediated decay.

This research is supported by the Intramural Research Program, National Heart, Lung, and Blood Institute, National Institutes of Health

428-C Identification of novel UPF1 target transcripts by direct determination of whole transcriptome stability.

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UPF1, an evolutionarily conserved protein, plays the central role in nonsense-mediated mRNA decay (NMD), which eliminates aberrant mRNAs harboring premature termination codon (PTC), preventing the accumulation of nonfunctional or potentially harmful truncated proteins. UPF1 is also involved in staufen1 (STAU1)-mediated mRNA decay (SMD) as well as replication-dependent histone mRNA decay. Thus, UPF1 is an important factor not only for the RNA quality control system but also for the regulation of physiological gene expression through regulation of RNA stability. In this work, we directly measured the stability of whole transcriptome in UPF1 knock-down HeLa cells by BRIC-seq method (5¢-bromo-uridine immunoprecipitation chase–deep sequencing analysis) that was developed by us. We successfully determined the half-lives of ~10,000 transcripts, and found that 785 transcripts were stabilized in UPF1 knock-down HeLa cells. Among 785 stabilized transcripts, the expressions of only 76 transcripts were increased and remaining 709 transcripts were not altered. RNA immunoprecipitation experiment showed that UPF1 bound to the transcripts whose decay was interfered but their expression was not changed, suggesting that UPF1 directly destabilizes 709 transcripts. Most of the transcripts identified as UPF1 targets in this study were not found in previous studies, therefore, we identify novel hundreds of UPF1 targets in HeLa cells. Moreover, we found that GC-rich sequence is statistically enriched among UPF1 target transcript, suggesting that UPF1 regulates the stability of mRNAs harboring GC-rich sequence.

431-C Expression of nonsense-mediated decay factors is controlled by conserved feedback loops and responds to salt stress in plants

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Nonsense-mediated decay (NMD) is a conserved eukaryotic mRNA surveillance pathway that selectively recognizes aberrant transcripts and targets them for degradation. NMD target transcripts can be generated by faulty gene expression or selectively produced to serve gene regulation. NMD is triggered by c*is*-acting elements, comprising premature termination codons (PTCs), upstream open reading frames (uORFs), long 3' untranslated regions (UTRs), and 3' UTR-located introns. Recent studies indicated that more than 10% of all genes from *Arabidopsis thaliana* produce at least one NMD transcript variant, suggesting a major role of NMD in shaping the plant transcriptome. However, it is unclear whether NMD activity is constitutive or can be altered under certain developmental or stress conditions. Here we present data suggesting feedback regulation of NMD factor expression in *A. thaliana*. The transcripts from three of the core NMD factors (UPF1, UPF3, and SMG7) contain long 3' UTRs and/or 3' UTR-positioned introns and are upregulated in NMD-impaired seedlings. Furthermore, transcript levels of these NMD factors are elevated upon salt stress in seedlings, which might be explained by diminished NMD activity under these conditions. In line with this, the transcript from another core NMD factor, UPF2, that lacks any NMD eliciting feature display unchanged expression upon both NMD impairment and salt stress. Interestingly, we found that both feedback and salt stress regulation of NMD factors is also present in *Nicotiana benthamiana*, revealing evolutionary conservation of NMD control in different plant species. Our data suggest that NMD activity might be significantly altered under certain conditions, which we currently further investigate by use of an *in vivo* NMD reporter system.

434-C SMG7 recruits the CCR4-NOT complex for degradation of NMD targets

Belinda Loh[1], Stefanie Jonas[1], Elisa Izaurralde[1]

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The nonsense-mediated mRNA decay (NMD) pathway triggers rapid degradation of aberrant mRNAs that contain premature translation termination codons (PTCs). In metazoans, NMD requires three 14-3-3-like proteins: SMG5, SMG6, and SMG7. These proteins are recruited to PTC-containing mRNAs through the interaction of their 14-3-3-like domains with phosphorylated UPF1, the central NMD effector. The recruitment of SMG5, SMG6, and SMG7 causes NMD target degradation. SMG6 possesses an active PIN domain at its C-terminus that cleaves the target mRNA in the vicinity of the PTC. On the other hand, tethered SMG7 has been shown to degrade mRNA efficiently through its Proline-rich C-terminus (PC) region, which is necessary and sufficient for this activity. Previous studies indicate that SMG7-mediated mRNA degradation requires the general mRNA decay enzymes. However, the mechanism by which these enzymes are recruited to the target mRNA has remained unclear. To determine how SMG7 elicits mRNA decay, we used the Tandem-Affinity Purification (TAP-tag) methodology to identify interacting partners. Amongst the SMG7-binding proteins, we found all subunits of the CCR4-NOT complex that are responsible for deadenylation of mRNAs. We further show that the PC-region of SMG7 is responsible for binding POP2, the catalytically active component of the CCR4-NOT complex. Additional mapping experiments of POP2 reveal that the catalytic domain alone is sufficient to bind the PC region of SMG7. Over-expression of catalytically inactive POP2 shows stabilization of SMG7-tethered RNA and PTC-containing reporter RNA. In addition, over-expression of a catalytically inactive DCP2, the decapping enzyme, also results in stabilization of NMD targets. Functional studies in human cells demonstrate that the PC region of SMG7 is required for NMD only in cells depleted of SMG6. Together with previously published data, our findings show that the 5' to 3' decay pathway is utilized in the SMG7-dependent degradation of NMD targets.

437-C Global analysis of exosome target introns

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U12-type introns are a class of rare introns in the genomes of diverse eukaryotes. They number over 800 introns in the human genome, making up less than 0.5 % of our introns (Turunen et al., 2013). These introns have earlier been shown to splice at a slower rate compared to the major U2-dependent pathway. This suggests a rate-limiting regulatory function for the minor spliceosome in the nuclear processing of transcripts containing U12-type introns (Patel et al. 2002). In support to this model, an elevated level of unspliced U12-type introns have been detected in the steady-state RNA populations in various organisms (Patel et al. 2002; Pessa et al. 2006; Pessa et al. 2010) However, both the mechanism of slower splicing kinetics and the fate of mRNAs containing unspliced U12-type introns remain unknown.

Here we have analyzed globally the effect of exosome processing on the nuclear pre-mRNA transcripts by inactivating either the Rrp41 or Dis3 subunit of the exosome. Using SOLiD RNA sequencing technology, we report 30-120 million mapped cellular compartment specific reads per sample allowing the detection of unspliced pre-mRNAs.

We show that Rrp41 and Dis3 knockdowns stabilize an overlapping set of U12-type introns, with a total of 132 stabilized introns using 2-fold upregulation as cutoff. At least 3-fold upregulated introns total 84, and at least 4-fold upregulated introns number 57, covering a remarkable fraction of the human U12 intron set (15 %, 9.5 % and 6.5 %, respectively). Collectively the median U12-type intron retention distribution shifts towards upregulation in the knockdown, contrary to U2-type introns, whose distribution shows no change. Validation of the top stabilized introns is performed using RT-PCR. Finally, we explore the characteristics of introns targeted by the exosome.

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Pessa HK, Ruokolainen A, Frilander MJ. (2006) The abundance of the spliceosomal snRNPs is not limiting the splicing of U12-type introns. RNA. 12:1883-92.

Pessa HK, Greco D, Kvist J, WahlstrÖm G, Heino TI, Auvinen P, Frilander MJ. (2010) Gene expression profiling of U12-type spliceosome mutant Drosophila reveals widespread changes in metabolic pathways. PLoS One. 5:e13215.

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440-C Structural insights into the Dhh1-Pat1 interaction

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Eukaryotic mRNA turnover starts with the shortening of the 3' poly (A) tail and continues with either 3'-5' degradation by the exosome complex or with decapping and 5'-3' degradation by Xrn1. Decapping is catalyzed by the Dcp1-Dcp2 complex and is regulated by several co-activator proteins, including Dhh1, Pat1, Edc3 and the heptameric Lsm 1-7 complex. All these factors are conserved from yeast to humans.

Pat1 is a multidomain protein. It uses the N-terminal domain to interact with Dhh1 and the C-terminal domain to interact with Lsm1-7. As such, Pat1 is believed to act as a bridging factor between the 3' end and the 5' end of the message. Dhh1 (also known as Rck or DDX6 in metazoans) is a DEAD-box protein. Like all other members of the DEAD-box family, Dhh1 is expected to bind RNA via the two RecA domains. In addition, Dhh1 interacts with another enhancer of decapping, Edc3. How Dhh1 interacts with Pat1 and how this affects the RNA-binding and protein-protein binding properties of this DEAD-box protein is unclear.

We have determined the 2.8 Å crystal structure of yeast Dhh1 bound to the N-terminal domain of Pat1. The structure reveals an evolutionary conserved recognition mechanism that we have confirmed by testing mutations in *in vitro* assays with the yeast proteins and in co-immunoprecipitation assays with the corresponding human orthologues. Comparison with the known structures of human DDX6-Edc3 and of DEAD-box proteins bound to RNA reveals how Pat1 binding impacts on other macromolecular interactions mediated by Dhh1.

443-C MKT1: a hub in a post-transcriptional regulatory network

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Trypanosomes are uniquely reliant upon post-transcriptional mechanisms for the control of gene expression. We previously found that stabilisation of trypanosome mRNAs during heat shock depends on binding of the zinc finger protein ZC3H11 to the 3'-untranslated regions of target mRNAs; also, tethering of ZC3H11 to a reporter mRNA increases reporter expression, suggesting an active stabilisation mechanism. We have now found that ZC3H11interacts with trypanosome MKT1 and PBP1, and that interactions with both proteins are required for ZC3H11 activity in the tethering assay. *Saccharomyces cerevisiae* Mkt1p facilitates yeast survival during stress, while yeast Pbp1p interacts with Lsm12p and the poly(A) binding protein Pab1p. Both Pbp1p and its human homologue, Ataxin-2, are stress granule components.

Trypanosome MKT1, like Mkt1p, interacts with PBP1 and LSM12, and trypanosome PBP1 interacts with trypanosome poly(A) binding proteins. MKT1, PBP1 and LSM12 are all essential for parasite survival, and all increase reporter expression in the tethering assay, as does poly(A) binding protein. MKT1, like Mkt1p, is polysome associated. MKT1 and PBP1 are distributed throughout the cytoplasm at the normal growth temperature and after heat shock, but concentrated in stress granules during starvation. Stabliisation of heat-shock mRNAs could result from cooperative interactions between ZC3H11, MKT1, PBP1 and poly(A) binding proteins, resulting in enhanced protection of the poly(A) tail against deadenylation.

A two-hybrid assay, analysed by deep sequencing, revealed that MKT1 interacts with at least 10 proteins with RNA-binding domains, some of which were also active in a high throughput tethering assay. There were, however, also possible interactions with the mRNA degradation machinery. We suggest, therefore, that trypanosome MKT1 is at the centre of a post-transcriptional regulatory network.

446-C Enriched density of UPF1 in 3’ untranslated regions results from its translation-dependent displacement from coding sequences

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The RNA helicase UPF1 is best known for its key function in mRNA nonsense-mediated mRNA decay (NMD), but has also been implicated in additional mRNA turnover mechanisms, telomere homeostasis, and DNA replication. In NMD, UPF1 recruitment to target mRNAs is thought to occur through interaction with release factors at terminating ribosomes, but evidence for translation-independent interaction of UPF1 with the 3' untranslated region (UTR) of mRNAs has also been reported. To map UPF1 binding sites transcriptome-wide, we performed individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) in human cells, untreated or after inhibiting translation by puromycin. Our results revealed a strongly enriched association of UPF1 with 3' UTRs in undisturbed, translationally active cells. After translation inhibition, a significant increase in UPF1 binding to coding sequence (CDS) was observed, indicating that UPF1 binds RNA before translation and gets displaced from the CDS by translating ribosomes. Our evidence for translation-independent UPF1-RNA interaction, which is corroborated by UPF1 crosslinking to long non-coding RNAs, suggests that the decision to trigger NMD occurs after association of UPF1 with mRNA, presumably through activation of RNA-bound UPF1 by aberrant translation termination.

RNA Editing and Modification

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 449 C – 473 C
Location: Main Hallway & Sanada Foyer

449 C	**Revealing the full scope of Alu editing - over a hundred million genomic sites are subject to primate-specific A-to-I RNA editing**
452 C	**Alu inverted repeats induce human specific site selective A-to-I RNA editing**
455 C	**RAM: a novel and essential component of RNA cap methylation**
458 C	**Consequences of FilaminA editing**
461 C	**A single-molecule study on the molecular mechanism of microRNA uridylation**
464 C	**Functional implications from the atomic model of the poly(U) polymerase Cid1**
467 C	**A Comprehensive Analysis of RNA Modifying Enzymes in Zebrafish**
470 C	**Engineered guideRNA-Dependent Deaminases - A Tool to Modify mRNA**
473 C	**Enzymes involved in human cap structure formation: their structure and function**

449-C Revealing the full scope of Alu editing - over a hundred million genomic sites are subject to primate-specific A-to-I RNA editing

Lily Bazak[1], Ami Haviv[1], Michal Barak[1], Jasmine Jacob-Hirsch[1], Karen Kesarcas[2], Gideon Rechavi[2], Jin Billy Li[3], Erez Y. Levanon[1], Eli Eisenberg[4]

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RNA molecules carry the information encoded in the genome and reflect its content. Adenosine-to-inosine modification of RNA molecules (A-to-I RNA editing) by ADAR proteins converts a genomically encoded adenosine (A) into inosine (I). It is known that most RNA editing in human take place in the primate specific Alu sequences but the extent of this phenomenon and its effect on transcriptome diversity is not clear yet.

Analyzing large-scale RNA-seq data and by performing ultra-deep sequencing of selected Alu sequences, we show that the scope of editing is much larger than was anticipated. More than 700,000 Alu repeats can form dsRNA structures, and virtually all adenosines within these Alu repeats undergo A-to-I editing to some extent. Moreover, we observe editing of transcripts resulting from residual anti-sense expression, doubling the number of edited sites in the human genome. The total number is thus estimated to exceed a hundred million sites. Our ultra-high coverage enables us to probe editing levels that span a wide range, with few sites being fully converted while most sites exhibit low (<1%) levels.

We further studied the effect of Alu editing on transcriptome diversity. The number of different variants seem to grow with coverage, with no sign of saturation. Looking at the information included in each Alu repeat in terms of its editing pattern, Shanon's information entropy ranges from 5 to 9 bits per Alu. The average number of inosines per transcribed Alu also varies considerably among the randomly selected Alus, ranging between 1 and 19 inosines per transcript. Finally, we estimate the number of Alu-derived inosines to be roughly 100,000-fold higher than the number of inosines located in the Q/R of the glutamate receptor.

These finding naturally lead to the question of how this primate-specific diversification of the transcriptome is utilized.

452-C Alu inverted repeats induce human specific site selective A-to-I RNA editing

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RNA editing by adenosine (A) deamination to inosine (I) is a common event in the human transcriptome due to the high frequency of *Alu* elements. *Alu* inverted repeats are targeted for A-to-I editing because of their ability to form hairpin structures of double stranded RNA, a requisite for ADAR editing. Recent high throughput sequencing (RNA-Seq) has revealed several hundred thousand edited sites within these *Alu* repeats. Nevertheless, the function of editing within repetitive elements is largely unknown. Using bioinformatic and experimental analyses we show that primate specific site selective editing in non-repetitive sequence often is found adjacent to *Alu* inverted repeats. We propose that *Alu* elements forming long, almost completely base paired structures can work as recruitment elements for the ADAR proteins and thereby induce editing in non-repetitive sequence. One primate specific site of editing is located in the transcript coding for the DNA repair enzyme NEIL1. Editing within exon 6 of this mRNA recodes a lysine for an arginine (K/R). We show that efficient editing at this site is dependent on the presence of a cis-acting *Alu* inverted repeat located 200 nucleotides upstream of the K/R site. We found this site to be efficiently edited also in rhesus monkey but not in mouse and rat. The upstream *Alu* inverted repeat in NEIL1 is conserved between rhesus and human. We propose that recruitment of ADAR to *Alu* elements increase the local concentration of the editing enzyme and thereby increase editing efficiency at other sites located in less stable duplex structures. We therefore suggest that *Alu* repeats can work as editing inducers, specifically increasing the variability in the human transcriptome.

455-C RAM: a novel and essential component of RNA cap methylation

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Eukaryotic gene expression is dependent on the 7-methylguanosine cap moiety, which is located at the 5' end of all RNA polymerase II primary transcripts. The 7-methylguanosine cap has a central role in most gene expression layers including transcription, splicing, nuclear export of both mRNA and snRNA, mRNA translation and decay and miRNA processing. The enzymes that catalyze the formation of the 7-methylguanosine cap are recruited to RNA polymerase II at the initial stages of transcription. The final step in this process, N-7 methylation of the guanosine cap, is catalyzed by the RNA guanine-7 methyltransferase, RNMT.

Recently, we demonstrated that RNMT does not function as a monomer but instead forms a heterodimer with a protein we designated as RAM (RNMT activating mini-protein), which is a novel component of the mammalian cap methyltransferase complex. The vast majority of cellular RNMT was found in a complex with RAM and *vice versa*. RAM is an RNA-binding protein, promoting recruitment of RNA to RNMT. RAM also increases recombinant and cellular RNMT cap methyltransferase activity and it is required for guanine-7 methylation *in vivo*. Therefore, we described RAM as an "obligate activator" of the human cap methyltransferase. As expected of a protein essential for cap methylation, RAM is required for mRNA translation, and loss of RAM results in loss of cell viability[1].

Here we dissect RAM functional domains and we demonstrate that RAM and RNMT can protect each other from proteasomal degradation. RAM N-terminus, which mediates RNMT interaction, is sufficient for stabilizing RNMT. However, the minimal RAM domain that is required for RNMT activation is more extensive than the minimal RAM domain that mediates the interaction with RNMT. This may suggest that upon RAM binding RNMT undergoes a major conformational change. Current studies focused on determining the crystal structure of RAM–RNMT complex will shed light on this hypothesis.

In addition, here we demonstrate that RAM C-terminus is essential for RAM nuclear accumulation. Although RNMT contains three classical nuclear localization signals (NLS) and utilizes the Importin-a/ß pathway for its nuclear import, RAM does not contain a classical NLS. Therefore, it was speculated that RAM is imported into the nucleus *via* RNMT interaction. However, the observation that RAM–RNMT interaction is not sufficient for RAM nuclear localization indicates that RAM contains its own NLS. Preliminary results suggest that RAM nuclear localization is mediated by Transportin-1, which recognizes a PY-NLS motif at RAM C-terminus. These data propose that RNMT and RAM utilize distinct nuclear import pathways and that the cap methyltransferase complex is only formed in the nucleus.

1. Gonatopoulos-Pournatzis, T., et al., RAM/Fam103a1 is required for mRNA cap methylation. *Molecular cell* **44**, 585–96 (2011).

458-C Consequences of FilaminA editing

Mamta Jain[1], *Maja Stulic*[1], *Dieter Pullirsch*[1], *Michael Jantsch*[1]

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RNA editing by ADARs (Adenosine deaminases that act on RNA) is a highly conserved phenomenon leading to diversification of the transcriptome. Of all RNA editing events, Adenosine to Inosine conversion is most common in metazoan, where it affects structured RNAs. A-I conversion occurs in many non coding sequences but is also found at highly conserved protein coding sites. One of such target encodes Filamin A (FLNA). Filamin A is an actin cross-linking protein known to be involved in a variety of functions including cell migration, cell adhesion and cell signaling. Filamin A is built of 24 Ig-fold repeats and editing at one site in repeat 22 leads to a single amino acid change (Glutamine to Arginine) in the protein. Although the function of Filamin A is quite well known, the significance of FLNA editing is poorly understood.

In order to understand the role of FLNA editing, we generated a mouse deficient in FLNA editing ($FlnA^{?ECS}$). Homozygous null mice are viable, fertile and show normal development. The behavioral and physiological phenotyping in these mice highlighted a significant difference in social discrimination and acoustic startle response. Using deep sequencing method from various mouse organs, we found that FLNA editing is highest in the gastrointestinal tract, heart and dorsal aorta. In agreement with FLNA's function as an actin organizing protein, we observed disorganized actin in the epithelial surface of stomach and large intestine of $FLNA^{?ECS}$ mice. Upon challenge with DSS (Dextran sodium sulfate) to induce colitis, our preliminary analyses demonstrate that $FlnA^{?ECS}$ mice show more severe inflammation as compared to controls. This suggests that FLNA editing could function to give protection against epithelial inflammation. Further studies would test the role of FLNA editing in the pathophysiology of colitis and its underlying mechanisms.

Parallel studies focusing on the effect of FLNA editing on cellular phenotypes highlighted its role in cell migration. $FlnA^{?ECS}$ mEFs show much reduced cell migration which was found to be matrix dependent as compared to controls. Currently, we are studying actin remodelling and Integrin signaling in these cells to understand the mechanism by which FLNA editing is involved in cell migration.

461-C A single-molecule study on the molecular mechanism of microRNA uridylation

Luuk Loeff[1], Kyu-Hyeon Yeom[3], Mohamed Fareh[1], V. Narry Kim[2], Chirlmin Joo[1]

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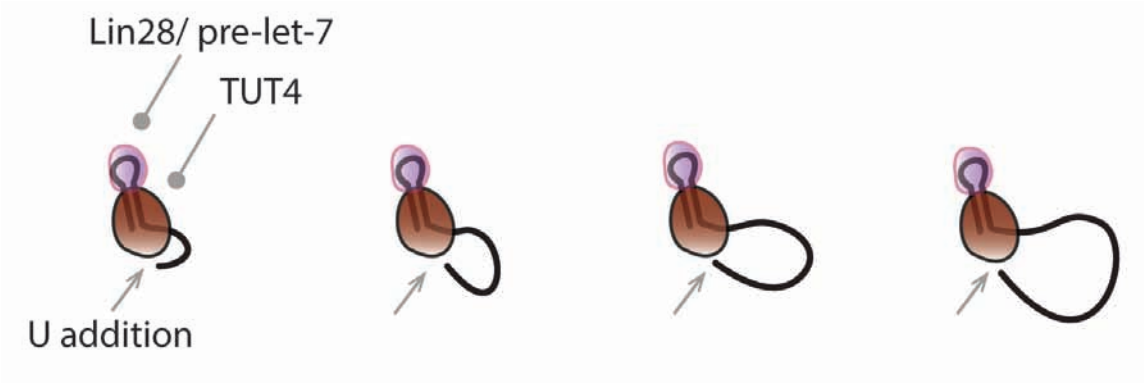
MicroRNAs are a class of short non-coding RNAs which play a key role in the regulation of gene expression in eukaryotes. Even though the birth and maturation of these small RNAs are well-characterized, the regulation and degradation pathways have only recently been explored. Previously, we and other groups showed that TUT4 (Terminal Uridylyl Transferase 4) uridylates precursor microRNA (pre-miRNA) in coordination with Lin28 and thus acts as a posttranscriptional repressor of microRNA maturation. Using single-molecule fluorescence spectroscopy, we show that Lin28 mediates a stable interaction between TUT4 and pre-miRNA [Ref-2011]. With FRET, we further show that TUT4 maintains the tight contact with pre-miRNA and Lin28, while it captures the 3' end of RNA and brings this to its catalytic domain. This mechanism leads to the formation of a unique closed loop of the U tail (see figure 1 below) [Ref-unpublished]. Our study provides insight into the molecular mechanism of Lin28-mediated oligo-uridylation. In addition, it may give a hint to a general mechanism of action of terminal uridylyl transferases.



464-C Functional implications from the atomic model of the poly(U) polymerase Cid1

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In eukaryotes, mRNA degradation begins with poly(A) tail removal, followed by decapping, and finally mRNA digestion by exonucleases. In recent years, the major influence of 3'end uridylation as a regulatory step within several RNA degradation pathways has driven attention toward the poly(U) polymerase (PUP) enzymes. The protein Cid1 is the prototype of the PUP enzyme found in higher eukaryotes. We have determined the atomic structures of the Cid1 protein bound to its substrate and to its minimal product. Point mutations highlight key residues implicated in the catalytic cycle. Our study further underlines the RNA binding properties of Cid1, in particular for the stabilization of the substrate RNA molecule. Functional characterization of these features are critical for our understanding of miRNAs, histone mRNAs and, more generally, for cellular RNA degradation.

467-C A Comprehensive Analysis of RNA Modifying Enzymes in Zebrafish

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The chemical modification of RNA nucleosides is a widespread phenomenon that occurs in all species analyzed so far. Some of these modifications are highly conserved throughout evolution and most enzymes required for their generation have been identified and characterized. Nevertheless, we are still lacking insights into the *in vivo* functions of most of these modifications. Interestingly, in lower eukaryotes, deletion of most RNA modification pathways is not essential. In contrast, deletion of RNA modifying enzymes in higher eukaryotes can lead to severe phenotypes. While most research has been performed in single cell organisms, we know only little about RNA modification pathways in vertebrates. Do RNA modification defects generally lead to more severe phenotypes in higher eukaryotes and are RNA modification enzymes differentially regulated during development and in different tissues?

To gain insights into the spatio-temporal regulation of RNA modification pathways, we undertook a comprehensive analysis of the expression patterns of RNA modification genes in zebrafish (*Danio rerio*) using *in situ* hybridization. To this end, we identified homologues of all known RNA modification genes in yeast and analyzed their expression patterns throughout zebrafish embryogenesis using embryos at 4h, 10h, 24h, 48h and 72h post fertilization (hpf).

Here we present the expression patterns of 56 putative RNA modification genes and knockdown experiments of selected candidates in zebrafish. We found, that expression levels of RNA modification genes differ. Furthermore, we observed an upregulation of RNA modifying genes during later embryogenesis (24hpf, 48hpf, 72hpf) in particular tissues: brain, eyes, branchial and pharyngeal arches, pectoral fin buds and myomeres. Our results provide insights into the developmental regulation of RNA modification genes in zebrafish and lay the basis for future investigations of the *in vivo* functions of RNA modifying genes in vertebrates.

470-C Engineered guideRNA-Dependent Deaminases - A Tool to Modify mRNA

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RNA modification is an important mechanism in higher organisms to alter gene expression and to diversify the gene products.[1] Modifications include pseudo-uridinylation, 2'-hydroxymethylation, and adenosine-to-inosine (A-to-I) editing. In contrast to other modifications, the effect of A-to-I editing is readily predictable since inosine is read as guanosine in biochemical reactions. Thus, A-to-I editing formally introduces an A-to-G point mutation on the RNA-level and results, for instance, in 1) the highly specific reprogramming of single amino acid codons, and 2) the alteration of RNA splice patterns.[1,2] Consulting the table of the genetic code one finds that 12 out of the 20 canonical amino acids could be targeted including Asp, Glu, Asn, Gln, His, Lys, Arg, Ser, Thr, Tyr, Ile, and Met/Start, and all three Stop codons, a striking accumulation of residues that are essential for enzyme catalysis, posttranslational modification (signaling), and general protein function. Consequently, harnessing enzymatic A-to-I deamination would make it possible to manipulate RNAs and their protein products in a currently unprecedented manner.

A-to-I editing normally operates via a protein-guided mechanism, thus its re-direction towards new targets has not been achieved yet. In our study,[3] we now present a simple way to steer deaminase activity towards user-defined sites on mRNA in order to introduce point mutations. To achieve the most rational and arbitrary target selection, we turned hADAR1 into a guide-RNA-dependent enzyme by covalently attaching a guide-RNA to its deaminase domain (see Figure). The potential and limitations of re-directing RNA-editing for application in biochemistry and medicine will be discussed.

References:

[1] K. Nishikura, *Annu. Rev. Biochem.* **2010**, *79*, 321.

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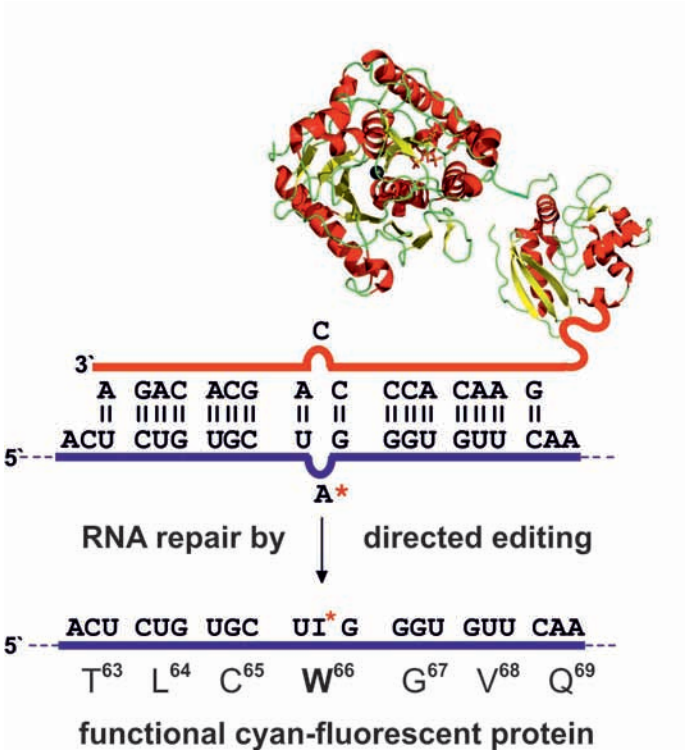

473-C Enzymes involved in human cap structure formation: their structure and function

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The 5' cap of human messenger RNA consists of an inverted 7-methylguanosine linked to the first transcribed nucleotide by a unique 5' - 5' triphosphate bond followed by 2'-O-ribose methylation of the first and often the second transcribed nucleotides, serving to modify efficiency of transcript processing, translation and stability. Enzymes that methylate ribose moieties of the first and the second nucleotide of the transcript, named hMTr1 and hMTr2 respectively, have been recently identified. Both enzymes contain related catalytic domains with a Rossmann-like fold characteristic for the RFM superfamily of methyltransferases (MTases), as well as additional domains. However their structures and mechanisms of substrate recognition and methylation remain unknown. We report the crystal structure of methyltransferase (MTase) domain of hMTr1 in an unliganded form, as a ternary complex with a cofactor S-adenosyl methionine (SAM) and a 5' mRNA cap analogue, and a complex with SAM and a short capped RNA. These structures reveal that with the exception of the methylated guanosine, the interactions occur between the protein and the phosphodiester backbone of the RNA molecule. This suggests that substrate binding and methylation are sequence–independent. Based on the crystal structure of hMTr1 catalytic domain as a template we generated a comparative model of the hMTr2 catalytic domain and carried out mutational analysis of hMTr2, which disclosed residues important for RNA and SAM binding. Inability to establish human somatic cells lacking *HMTR2* coding sequence argues for an essential function of its protein product in cell metabolism.

Splicing Mechanisms

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 476 C – 488 C
Location: Main Hallway & Sanada Foyer

476 C	**Biophysical characterization of the recombinant S. cerevisiae Lsm2-8 complex**
479 C	**Conserved Slu7 motif confers the preference for distal splice sites in yeast**
482 C	**A new role for U2 snRNA in alternate 3' ss selection**
485 C	**Identification of small molecule pre-mRNA splicing inhibitors using a stage-specific, high-throughput in vitro splicing assay**
488 C	**The G patch protein Spp2 couples Prp2-mediated ATP hydrolysis to catalytic activation of the yeast spliceosome**

476-C Biophysical characterization of the recombinant S. cerevisiae Lsm2-8 complex

Elizabeth Dunn[1], Trushar Patel[3], Richard Fahlman[2], Calvin Yip[5], Sean McKenna[3], Stephen Rader[4]

[1]Department of Biochemistry and Molecular Biology, University of British Columbia; [2]Department of Biochemistry, University of Alberta; [3]Department of Chemistry, University of Manitoba; [4]Department of Chemistry, University of Northern British Columbia; [5]Life Sciences Institute, University of British Columbia

Biochemical and biophysical studies of macromolecules have historically focused on simple systems, frequently monomers or dimers of proteins. Many of the most important cellular processes, however, are carried out by complicated, multicomponent assemblies. A prime example of this is the spliceosome, the nuclear complex that carries out pre-mRNA splicing, which in *S. cerevisiae* contains five small, nuclear RNAs (snRNAs) and nearly 100 proteins. While the study of such complex systems has taken on increased urgency as their importance has become clearer, their recombinant expression and purification remain challenging.

In this poster, we report the development of a method to express and purify the hetero-heptameric LSm complex of proteins that associates with U6 snRNA. U6 is the most highly conserved snRNA, and substantial evidence suggests that it is intimately involved in the catalytic steps of splicing. The investigation of U6 function is complicated by its highly dynamic nature: it exists by itself as the U6 small, nuclear ribonucleoprotein (U6 snRNP), as a base-paired complex with U4 in several particles, and as a base-paired complex with U2 in the active spliceosome. The U6-associated Lsm proteins are known to play a role in base pair formation with U4, and, intriguingly, they have been shown to dissociate from U6 prior to spliceosome activation, suggesting they may have additional functions.

We present a system for simultaneously expressing all seven U6-associated Lsm proteins recombinantly in *E. coli*. The proteins appear to express at approximately stoichiometric levels, and co-purify as a single peak by gel filtration chromatography. Mass spectrometry confirms the presence of all seven proteins in the complex, and electron microscopy demonstrates that they assemble into toruses, as predicted from previous studies. Ab initio models of the molecular envelope based on SAXS data are consistent with crystal structures of homologous protein complexes. We are currently characterizing the ability of the Lsm complex to associate with U6 snRNA and Prp24. Interestingly, the complex appears to dramatically promote the association of U4 and U6, even in the absence of any other proteins. We anticipate that this system will be useful for the expression and analysis of numerous other protein complexes.

479-C Conserved Slu7 motif confers the preference for distal splice sites in yeast

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Mechanisms governing proper 3' splice site (3' ss) selection for the 2nd catalytic reaction of splicing, which are important for alternative 3' ss usage in Metazoans, are only incompletely understood. In *S. cerevisiae*, sequence specific (re)positioning of 3' ss for catalysis occurs only after lariat formation.

Splicing in *S. cerevisiae* represents a unique system to examine the evolutionarily conserved splicing factors in a simplified setting. One of the key factors that is necessary for 3´ ss positioning is the essential 2nd step factor Slu7, which associates with the spliceosome only immediately before or during the 1st step. Slu7 was found in vitro to be required for splicing of introns with 3´ ss located >9 bp from BP (Brys and Schwer, 1996). Allele slu7-1, isolated through its synthetic lethality with U5 mutants, suppressed the usage of distal splice sites in recombinant splicing substrates (Frank and Guthrie, 1992). Slu7 contains several highly conserved regions, but its N-terminal 199 amino acids, including a putative zinc knuckle motif (aa 122-135), are dispensable for the essential function (Zhang and Schwer, 1997).

Using the screen for synthetic lethality and subsequent direct testing, we identified genetic interactions between the allele of the splicing factor *PRP45* (*prp45*(1-169)) and several mutations of *SLU7* (Gahura et al. 2009). The mutations mapped between arginines 247 and 271 within the essential part of *SLU7*. Analyses of splicing *in vivo* revealed second step splicing defects and impaired utilization of suboptimal splicing substrates. Importantly, several point mutations suppressed the utilization of distant splice site when two competing sites were present on a recombinant substrate. This feature was most prominent when the proximal splice site was close to BP (~10 bp). The novel *SLU7* mutations also shifted the utilization of cryptic splice sites on an endogenous gene in favor of the proximal AG. The newly identified Slu7 motif, unlike, e.g., the zinc knuckle motif, is part of the structure that is responsible for efficient positioning of distal splice sites for catalysis. More extensive mutational analysis of this region is under way to elucidate the role that this part of the protein plays in pre-mRNA splicing.

This work was co-financed by the European Social Fund Project no. CZ.1.07/2.3.00/30.0022, MSM0021620858, and the Charles University grant SVV265211.

482-C A new role for U2 snRNA in alternate 3' ss selection

Jorge Pérez-Valle[1], Josep Vilardell[2]

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We have documented how several sequence features in the intronic region downstream from the BS act together to define the 3' ss. Briefly, the yeast spliceosome will pick any HAG (H = A, C, G) present in a window of ~35 nt, starting at position 10 from the BS; and because stems, loops, and bulges are not included in this window, pre-mRNA folding plays a critical role in 3'ss selection. Interestingly, this does not apply to the first nt after the BS; and instead our results are more consistent with the requirement to have this region unfolded for efficient splicing. They show that in a reporter with two 3'ss, one blocked by a stem and the other within spliceosomal reach, a switch in 3'ss selection can be induced by placing the stem at 10 nt or less from the BS. According to our model, a stem near the BS will not be formed, and this would both make available the occluded AG and place the downstream AG outside the spliceosomal window. The "exclusion zone" downstream from the BS would be also consistent with the proposed interaction of a number of essential spliceosomal factors with positions downstream the BS. However the molecular reasons for this remain unclear.

A genetic screen has revealed a role for U2 snRNA in modulating this 3'ss selection switch. Thus, in cells expressing U2 U40C the requirement for the unfolded region downstream from the BS is reduced and the 3'ss switch described above takes place closer to the BS. Position U40 is part of the U2 BSL stem-loop, which has been shown to play a role in BS identification (Perriman & Ares (2010) Mol. Cell). U40C stabilizes the BSL and promotes BS recognition as reported. However, other mutations that also stabilize the BSL, such as U44A, and U40G G32C, do not reproduce the U40C effect on 3' ss selection.

We have analyzed the intronic requirements for the U2-U40C phenotype and our data are consistent with the shifted recognition of the BS, by three nucleotides, for the first step of splicing. This shift occurs as well with the wt U2, albeit to a lesser extent. Surprisingly, with either U2 wt or U40C the shift is independent of having a stem near the BS. We are investigating the role of the regular BS in this process, as well as the molecular reasons behind the U2-U40C phenotype, distinct from other mutations that stabilize the BSL.

485-C Identification of small molecule pre-mRNA splicing inhibitors using a stage-specific, high-throughput in vitro splicing assay

Anzhela Sidarovich[1], Timur R. Samatov[1], Sonja Sievers[2], Cindy L. Will[1], Henning Urlaub[1], Herbert Waldmann[2], Reinhard Lührmann[1]

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Disruption/misregulation of alternative and constitutive splicing are the cause or a severity modulator of many human diseases, including among others cancer and, neurodegenerative and autoimmune diseases, making the spliceosome a highly attractive drug target. Small molecule inhibitors that block discrete steps of the extremely dynamic functional cycle of the spliceosome would not only be of potential therapeutic value, but also be highly useful for the detailed investigation of the structure and function of the spliceosome. However, only a limited number of small molecule inhibitors that specifically target the pre-mRNA splicing machinery have been identified to date. We previously established a robust, rapid and sensitive high throughput in vitro splicing assay, which monitors in the wells of a microplate the formation of step I spliceosomes (i.e. the spliceosomal C complex) by measuring the association of a FLAG-tagged version of the DEAD box ATPase, Abstrakt, which is incorporated into the spliceosome first upon C complex formation[1]. Using this assay, we screened a chemical library of approximately 160,000 compounds at 50 μM concentration. All compounds showing at least a 50% decrease in the signal intensity of bound Abstrakt were subjected to a second test, and reproducible hits were finally tested in an in vitro splicing assay using ^{32}P-labeled adenovirus-derived MINX-MS2 pre-mRNA as substrate. Ten compounds were confirmed to be inhibitors of pre-mRNA splicing in vitro, and exhibited IC_{50} values ranging from 3 to 50 μM. To determine at precisely which stage they inhibit splicing, we performed a splicing time course and analysed the spliceosomal complexes formed by native agarose gel electrophoresis. At least one compound (hereafter designated 28), led to an accumulation of A complexes and a complex migrating slightly faster than the B complex. To characterize in more detail spliceosomal complexes formed in the presence of compound 28, we subjected the stalled splicing reactions to glycerol-gradient centrifugation and purified the complexes in a given peak by MS2-MBP affinity selection. Initial analyses of the RNA and protein composition of complexes affinity-purified from the "B-like" peak, suggest that compound 28 may stall splicing at a novel stage of the spliceosome activation step. This apparently unique property may allow us to obtain a novel snapshot of the spliceosome assembly pathway for detailed structural and functional investigations, and thus to improve our limited understanding of the dynamic rearrangement of spliceosomal components during spliceosome activation.

[1]Samatov et al., Chembiochem. 2012, 13:640-644

488-C The G patch protein Spp2 couples Prp2-mediated ATP hydrolysis to catalytic activation of the yeast spliceosome

Zbigniew Warkocki[1], Jana Schmitzová[1], Claudia Höbartner[2], Patrizia Fabrizio[1], Reinhard Lührmann[1]

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The spliceosome is a dynamic molecular machine that is continuously remodeled during its assembly and catalytic cycle. A major structural rearrangement occurs during the transition from the activated B^{act} complex to the catalytically competent B* complex, which entails the loss and destabilization of numerous spliceosomal proteins. This structural rearrangement is driven by the DEAH-box ATPase Prp2 in cooperation with its essential co-factor Spp2. Basic questions concerning the requirements for triggering Prp2's ATPase activity and how Prp2-mediated ATP hydrolysis is coupled to the B^{act} to B* complex transition have not been answered yet. The role of the G patch protein Spp2 in this process is also not well understood. It has been shown, however, that Spp2 may interact in solution with the C-terminal DUF domain of Prp2 via its G patch region (MCB 24, 2004,10101). Here we have addressed these questions using a purified yeast splicing system (NSMB 16, 2009, 1237).

The major findings are as follows: (1) Spp2 significantly enhances the oligo(U)30-stimulated, but not the unstimulated, intrinsic ATPase activity of Prp2 in solution. (2) Prp2 binds stably to purified B^{act} spliceosomes in the absence of Spp2 and vice versa. The stability of Spp2 binding to the B^{act} complex is enhanced in the presence of Prp2, suggesting that they interact with each other in the spliceosome. (3) We have measured for the first time the spliceosome-dependent NTPase activity of Prp2 (using UTP instead of ATP as exogenous source of energy to avoid measuring possible ATPase activities of other endogenous ATPases such as Brr2) and show that B^{act} spliceosomes efficiently trigger Prp2-mediated UTP hydrolysis in the absence of Spp2, with an initial hydrolysis rate of about 120 UTP•Prp2-spliceosome^{-1}•min^{-1}, which does not significantly level off after 10 min. However, B^{act} spliceosomes do not undergo catalytic activation under these conditions. (4) In the presence of Spp2, Prp2 in the spliceosome exhibits a similar initial rate of UTP hydrolysis, which levels off after 5-7 min. This effect is even more pronounced in the presence of both Spp2 and Cwc25, suggesting that in the presence of the latter factors, the spliceosome is remodeled and looses its binding site for Prp2.

In conclusion, our data suggest that certain structural elements of the B^{act} spliceosome can efficiently trigger the NTPase activity of Prp2 even in the absence of Spp2. UTP hydrolysis by Prp2 in the spliceosome observed in the absence of Spp2 is, however, unproductive in that it does not lead to catalytic activation of the B^{act} spliceosome. The energy of Prp2-mediated UTP hydrolysis can only be coupled to structural rearrangements of the spliceosome that yield the B* complex when Spp2 is simultaneously present in the spliceosome.

Splicing Regulation

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 491 C – 527 C
Location: Main Hallway & Sanada Foyer

491 C	**TNF influences alternative stop codon usage in DAPK mRNA**
494 C	**Alternative splicing in the regulation of the barley circadian clock**
497 C	**H3K9me3 and its role in pre-mRNA splicing.**
500 C	**Alternative splicing and gene expression in cardiomyocytes and cardiac fibroblasts during development**
503 C	**Prp45 affects early stage of spliceosome assembly and pre-mRNA abundance in yeast**
506 C	**Elucidation of UP1 binding to RNA substrates: Does RNA structure matter?**
509 C	**Real-time kinetics of human pre-mRNA splicing**
512 C	**A highly conserved GC-rich element regulates alternative splicing of mRNA for the variant thyroid hormone receptor TRα2**
515 C	**Comprehensive Mapping of the Splicing Regulatory Circuitry Involved in Cell Proliferation and Apoptosis**
518 C	**hnRNP A1 promotes exon 6 inclusion of apoptotic Fas gene.**
521 C	**A Role for the Polyadenosine Binding Protein, Nab2, in Splicing and Quality Control**
524 C	**SWI/SNF regulates alternative trans-splicing of the mod(mdg4) gene**
527 C	**THE RIBOSOME-OME II: ALTERNATIVE SPLICING FOR RIBOSOMAL PROTEINS?**

491-C TNF influences alternative stop codon usage in DAPK mRNA

Natalya Benderska[1], Stefan Stamm[2], Regine Schneider-Stock[1]

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Death-associated protein kinase (DAPK) pre-mRNA is undergoing alternative splicing leading to the production of two isoforms, which elicit antagonistic functions. DAPKa mediates induction of apoptosis whereas the DAPKß isoform, extended by ten amino acids on the C-terminus, inhibits cell death. Until now nothing is known about the splicing elements regulating the introduction of an alternative stop-codon in the DAPK pre-mRNA. External signals leading to disproportion of DAPK transcripts are unknown as well. Therefore, this study aims to initiate the investigation of the complex regulatory mechanisms of DAPK splicing. We thus started to determine the regulatory mechanism of DAPK splicing.

To investigate the functional role of these isoforms in inflammation process we generated U937T macrophage cell lines stably expressing human DAPKa or DAPKß. DAPKa over-expression in differentiated macrophages led to a significant increase (up to 3-fold) in apoptosis accompanied by a significant decrease in TNF secretion. Although DAPKß over-expression supressed TNF secretion by macrophages the ß-isoform does not enforce apoptosis. Interestingly, preliminary work demonstrated different pattern of a and ß DAPK mRNA expression in normal and malignant intestinal epithelial cells. From these findings we suggest that a change in alternative splice site usage seems to be caused by cellular stress induced by cytokine over-production. To further study the role of TNF in DAPK alternative splicing we generated a DAPK minigene containing a retained intron inside of exon 26. TNF treated HEK293 cells transfected with the DAPK minigene showed a promotion of DAPKa isoform containing an intron retained sequence. Screening of splicing factors potentially involved in alternative stop-codon usage is ongoing.

This study will provide important information about possible triggering of DAPK isoforms in inflammatory and apoptotic pathways.

494-C Alternative splicing in the regulation of the barley circadian clock

Cristiane Calixto[1], *Robbie Waugh*[2], *John Brown*[1]

[1]Division of Plant Sciences, University of Dundee at The James Hutton Institute, Scotland; [2]Cell and Molecular Sciences, The James Hutton Institute, Scotland

The circadian clock is a cellular mechanism able to organize several physiological processes in anticipation/preparation to daily changes. As sessile organisms, plants strongly rely on the circadian clock to match processes such as leaf movement, immune responses and flowering according to the right time of the day/season. This 'internal clock' is maintained and run by clock genes and most of what is known about them in plants comes from studies in *Arabidopsis.* Regulation of the circadian clock in plants is complex involving interlocked expression feedback loops, control of protein phosphorylation and degradation, and chromatin remodelling. Recently, extensive alternative splicing (AS) was shown to regulate clock genes through dynamic changes in AS transcripts, some of which are temperature-dependent and altered levels of productive mRNAs through AS/NMD [1]. In particular, low temperatures reduced the levels of LHY transcript and protein without affecting the promoter strength, which suggests that its expression is regulated post-transcriptionally by AS events that produce nonfunctional transcripts. In addition, temperature-dependent AS had opposite effects on pairs of partially redundant clock components (LHY/CCA1; PRR7/PRR9). This raised the question of whether clock genes and their modes of regulation are conserved in other higher plants, such as crop species. We have now carried out a robust *in silico* analysis using 27 *Arabidopsis* core clock/clock-associated genes and have identified 21 barley genes, 60% of which are true *Arabidopsis* orthologues. Most of the barley clock genes have a clear daily rhythm which is maintained in constant light conditions. Mutations of the barley clock genes *HvPpd-H1* (a putative orthologue of *AtPRR7*) and *HvElf3* strongly affect flowering time (earliness) and have extended the geographic range where barley is grown. We show that both mutations affect expression of clock genes involved in flowering time: the *Hvppd-H1* mutation affects expression levels and phase while the *Hvelf3* mutation also causes arhythmicity of some of these genes which helps to explain their earliness. We have identified AS in some of the core orthologues and are addressing temperature-specific AS in barley. This novel layer of fine clock control observed in two different species, a model plant and a crop species, might help our understanding of plant adaptation in different environments and ultimately may offer a new range of targets for plant improvement.

[1] James et al (2012) Plant Cell 24: 961-981

497-C H3K9me3 and its role in pre-mRNA splicing.

Eva Duskova[1], Martina Huranova[2], Fernando Carrillo Oesterreich[3], David Stanek[1]

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Splicing of the pre-mRNA occurs mainly co-transcriptionally. Close relationship between transcription and splicing was recently extended to chromatin modifications, which affect splicing outcome. Our bioinformatics analysis revealed specific enrichment of H3K9me3 across splicing sites on actively transcribed genes. To study the impact of H3K9me3 on co-transcriptional splicing we prepared inducible stable cell lines expressing methyltransferase and demethylase. We monitor changes of H3K9me3 on selected gene and we analyze splicing efficiency. Our results suggest role of H3K9me3 in definition of splice sites and efficiency of co-transcriptional splicing.

500-C Alternative splicing and gene expression in cardiomyocytes and cardiac fibroblasts during development

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Alternative splicing (AS) expands the proteomic complexity in mammals. During postnatal development the fetal heart converts to adult function through transcriptional and posttranscriptional mechanisms, including AS regulation. The RNA binding proteins CELF1 and MBNL1 coordinately regulate AS during heart development affecting >40% of the developmental transitions: CELF1 is down regulated 10-fold after birth; MBNL1 is up regulated 4-fold. Heart tissue is ~20% cardiomyocytes (CMs), ~66% cardiac fibroblasts (CFs), epithelial and vascular cells. CMs provide contractility through excitation contraction coupling (ECC) and CFs provide a mechanical scaffold, electrophysiological conduction and signaling through paracrine function and direct CF-CM communication.

AS and gene expression transitions were studied by RNA-seq from total hearts of embryonic day 17, postnatal day 1 (PN1), 10, 28 and adult mice (>150 million paired-end reads, >80% genome alignment per sample). AS events identified by RNA-seq correlated well with RT-PCR validation (r^2=0.8). Gene ontology (GO) analysis revealed different enriched categories between developmental stages: protein transport and endocytosis between PN1-PN28; transcription and chromatin modifications between PN28-adult. Among CELF1 / MBNL1 dependent AS, endocytosis was an enriched functional target.

We also isolated CMs and CFs for RNA preparation within 3 hours from PN1-3, PN28-30, and adult mice for RNA-seq (>160 million paired-end reads, >84% genome alignment per sample). While CM-up regulated genes were enriched in mitochondrial metabolism categories, similar processes were enriched in CF-down regulated genes. A similar connection was found between CM-down regulation and CF-up regulation (cytokines, adhesion, JAK-stat and MAPK signaling) consistent with CMs-CFs communication *in vivo.* In terms of AS, more transitions were found in CMs (999) than in CFs (409). Interestingly in CMs 30% responded to MBNL1, 27% to CELF1 and between these 2 groups there was >50% overlap. Similarly with total heart, endocytosis was enriched in CMs suggesting a role of AS in vesicular traffic, membrane organization and invagination. Possibly this enrichment reflects: *i)* ligands/growth factors uptake changes, *ii)* surface density of ion channels changes, *iii)* the deep architecture reorganization involved in the postnatal maturation of sarcoplasmic reticulum and T-tubules which are crucial for ECC.

503-C Prp45 affects early stage of spliceosome assembly and pre-mRNA abundance in yeast

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Prp45 is an evolutionarily conserved essential splicing factor that was found to be part of B, C, and post catalytic spliceosomal complexes; Prp45 is believed to enter the splicing cycle as part of the Prp19 complex (NTC). Previously, we reported that the C-terminal part of Prp45 regulates the partition of the second step helicase Prp22 in Cwc2 pull-downs and affects the fidelity of 3' splice site choice in *S. cerevisiae*. Using substrates non-conforming to the consensus, we also found that branch site mutations decrease 1st step efficiency in *prp45*(1-169) cells relative to WT (Gahura et al., 2009).

Our recent data indicate that Prp45, besides its role in the 2nd splicing step, affects spliceosome function also before the first transesterification. We found that truncation of Prp45 (*prp45*(1-169)) resulted in pre-mRNA accumulation of intron containing genes but that the corresponding mRNA levels remained unchanged with the exceptions of long intron genes such as *COF1* and *IMD4*. We employed Mer1-dependent reporter substrates, SpR and ExR, which yielded translated products when either spliced or not spliced, respectively. Cells expressing truncated Prp45 displayed SpR/ExR ratios which were consistent with a defect before the first transesterification. We also examined Mer1-dependent pre-mRNA and mRNA accumulation of *MER2* and other endogenous meiotic genes. *prp45*(1-169) cells accumulated ~three times higher levels of *MER2* pre-mRNA than WT cells only when Mer1 was present (i.e., spliceosome was assembling on the pre-mRNA). ChIP experiments also revealed the impaired recruitment of Prp45(1-169)-HA to intron containing genes. Co-transcriptional association of other splicing factors with intron containing genes in prp45(1-169) cells was differentially affected, suggesting that Prp45 plays role before the B complex formation. Notably, we found earlier that *S. pombe* U2AF35, which forms part of early spliceosome and pre-dates NTC in the splicing cycle, interacts with Prp45 homolog SNW1 (Ambrozkova et al., 2001).

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506-C Elucidation of UP1 binding to RNA substrates: Does RNA structure matter?

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The RNA binding protein hnRNP A1 performs numerous functions: mRNA transportation, splicing regulation, translation initiation, and telomere biogenesis. Many infectious agents utilize hnRNP A1 for its RNA binding capabilities. Two such agents are HIV-1 and Enterovirus 71 (EV71). In HIV-1 infection, hnRNP A1 acts as a *trans* splicing regulator. Meanwhile, in EV71, hnRNP A1 stimulates IRES-mediated translation. These functions of hnRNP A1 could provide targets for small molecules which block the binding of hnRNP A1 to viral RNA.

In our studies of hnRNP A1 binding to RNA, sub-domains of larger constructs are investigated. The DNA unwinding protein UP1, which consists of the two RRM domains of hnRNP A1, is being used in all our experiments. The RNA constructs used are SL3 (ESS3) of HIV-1 splice site A7 and SLII of the EV71 IRES. Previous experiments have revealed both stem loops bind UP1.

By working with RNA constructs of different structure, we hope to determine if RNA structure is a significant factor for UP1 recognition. Previous studies have shown UP1 binds UAG sequences in single stranded RNA. Our studies have shown UP1 binds ESS3 at its hairpin loop. SLII has two binding sites, a hairpin loop and a 5 nucleotide bulge which contains a UAG sequence. Examination of UP1 binding to its RNA substrates has been done by NMR and ITC. NMR has shown residues in UP1 potentially involved in RNA binding. ITC experiments have revealed the thermodynamic profile of UP1 binding to SLII and ESS3.

509-C Real-time kinetics of human pre-mRNA splicing

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We have developed a system to visualize splicing in real-time in living human cells. We combined genomic integration of a single reporter gene in human cells, intron labelling with the MS2 technique and spinning disk confocal microscopy to directly image the kinetics of intron excision from pre-mRNA. The fluorescence intensity associated with a single transcription site, which appears as a diffraction-limited object, is quantified as a function of time. Increments in the fluorescence signal result from de novo transcription of MS2-binding sites, and its disappearance reflects intron excision. Fluctuations in fluorescence intensity are used to determine the intron lifetime. We also determined the number of introns present at each individual transcription site at any given time point based on the number of GFP molecules bound to intronic MS2-stem loops. The results reveal different splicing kinetics depending on intron length and splice site strength. These results have important implications for mechanistic understanding of splicing regulation in the live-cell context.

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

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In mammals the THRA gene is alternatively spliced and encodes two proteins: TRα1, the a-thyroid hormone receptor, which is widely expressed in all vertebrates; and TRα2, a non-hormone binding variant present only in eutherian mammals. Coding sequences unique to TRα2 share an antisense overlap with those of Rev-erba (NR1D1), a nuclear receptor and core component of the mammalian circadian clock. Intronic and exonic splicing enhancers for TRα2 have evolved in the context of sequences required for expression of Rev-erba mRNA. Of particular interest is a GC-rich region (designated G30) at the 5' end of the terminal exon that encodes the unique C-terminal sequence of TRα2 mRNA. This sequence is antisense to the 3'UTR of Rev-erba and tightly conserved in all eutherian Rev-erba mRNAs, but not in those from marsupials or monotremes that do not express TRα2. Closely spaced deletions and substitutions within G30, differing by as little as a single nucleotide, have dramatically different effects on TRα2 splicing depending on their precise position. For example, deletion of 12 nucleotides can lead to >95% inhibition or >2.5-fold enhancement of TRα2 splicing. Substitutions of 2 or more nucleotides within G30 also display a range of effects. Our results suggest that G30 is a highly structured sequence, possibly a G-quadruplex, and part of a larger complex splicing regulatory element that exerts both positive and negative effects on TRα2 expression. Factors bound to G30 may interact directly with the 3'splice site and polyadenylation site that define the terminal exon. Alternatively, this element may affect splicing via effects on transcription within this bidirectionally transcribed region. Further experiments to distinguish between these possibilities are underway.

515-C Comprehensive Mapping of the Splicing Regulatory Circuitry Involved in Cell Proliferation and Apoptosis

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We present an integrated experimental and computational approach aimed to derive a comprehensive functional interactions map of the Splicing Regulators involved in cell proliferation and apoptosis. Our strategy is based on screening the effects of knock down (KD) of 300 individual genes encoding factors implicated in the splicing process and its regulation on 37 selected targets. The target list encompasses alternative splicing events implicated in tumor progression including apoptotic regulators, signaling molecules and metabolic enzymes. Splicing output of the targets upon KD is robustly quantified by high-throughput capillary electrophoresis. In turn this information is used as input for state-of-the-art methods for Graphical Model Selection and Analysis in order to recover the structure of the underlying splicing regulatory circuitry and to identify distinct modules within its topology.

Our method accurately captures well-established functional associations demonstrating its validity as a general tool for mapping out the splicing regulatory landscape. We are further able to identify several novel associations and to differentiate between those critical for general or alternative splicing regulation. One compelling feature of our approach is the possibility to extend the screening to physiological / pharmacological treatments in order to link their effects to the splicing regulatory circuitry that underlies cell proliferation and apoptosis. As a proof of principle we map the associations of splicing-arresting drugs and iron-homeostasis to the functional network of splicing regulators.

518-C hnRNP A1 promotes exon 6 inclusion of apoptotic Fas gene.

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Fas is a transmembrane cell surface protein recognized by Fas ligand (FasL). When FasL binds to Fas, the target cells undergo apoptosis. A soluble Fas molecule that lacks the transmembrane domain is produced from skipping of exon 6 encoding this region in alternative splicing procedure. The soluble Fas molecule has the opposite function of intact Fas molecule, protecting cells from apoptosis. Here we show that knockdown of hnRNP A1 promotes exon 6 skipping of Fas pre-mRNA, whereas overexpression of hnRNP A1 reduces exon 6 skipping. Based on the bioinformatics approach, we have hypothesized that hnRNP A1 functions through interrupting 5' splice site selection of exon 5 by interacting with its potential binding site close to 5' splice site of exon 5. Consistent with our hypothesis, we demonstrate that mutations of the hnRNP A1 binding site on exon 5 disrupted the effects of hnRNP A1 on exon 6 inclusion. RNA pull-down assay and then western blot analysis with hnRNP A1 antibody prove that hnRNP A1 contacts the potential binding site RNA sequence on exon 5 but not the mutant sequence. In addition, we show that the mutation of 5' splice site on exon 5 to a less conserved sequence destructed the effects of hnRNP A1 on exon 6 inclusion. Therefore we conclude that hnRNP A1 interacts with exon 5 to promote distal exon 6 inclusion of Fas pre-mRNA. Our study reveals a novel alternative splicing mechanism of Fas pre-mRNA.

521-C A Role for the Polyadenosine Binding Protein, Nab2, in Splicing and Quality Control

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Gene expression is temporally and spatially regulated to produce a precise protein expression profile that dictates the function of each cell. From the onset of transcription, RNA binding proteins immediately associate with a nascent mRNA transcript and guide it through post-transcriptional processing events like 5'-capping, splicing, and 3'-end processing. Many messenger ribonucleoproteins (mRNP) that contact the mRNA transcript throughout its post-transcriptional journey have more than one role in mRNA biogenesis to ensure proper processing of an mRNA transcript and avoid production of faulty proteins. These processing steps are highly conserved with much of the mechanistic information gleaned from studies in budding yeast. The essential *S. cerevisiae* zinc-finger nuclear polyadenosine RNA binding protein, Nab2, has been implicated in control of poly(A) tail length and mRNA export; however, a role for this protein in splicing has not been explored. To globally examine splicing of endogenous intron-containing transcripts, we performed a splicing microarray using *nab2* mutant cells. A mild constitutive splicing defect was detected in these *nab2* mutant cells. Consistent with a role for Nab2 in modulating splicing, we also detected defects in splicing in an *in vitro* splicing assay. To begin to understand which splicing step depends on Nab2, we tested for genetic interactions between *NAB2* alleles and splicing factors required at different steps in splicing. We observed synthetic lethality with splicing factors required early in splicing. A physical interaction was confirmed between Nab2 and the commitment complex proteins, Mud2 and Msl5, which are critical for both splicing and nuclear retention of unspliced transcripts. We then utilized a novel GFP reporter to track splicing and retention in Nab2 and Mud2 double mutants and found an exacerbated splicing defect as well as a reduction in pre-mRNA leakage. Alleles of *NAB2* also genetically interact with the mRNA decay machinery, suggesting that this atypical polyadenosine RNA binding protein acts as a nuclear watchdog to prevent accumulation and export of aberrant unspliced mRNAs. Our findings identify a role for Nab2 in regulating splicing and offer insights into how splicing and quality control are coupled in *S. cerevisiae*.

524-C SWI/SNF regulates alternative trans-splicing of the mod(mdg4) gene

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Trans-splicing is a pre-mRNA maturation event by which two pre-mRNA molecules are spliced together forming a processed mRNA. In this study, we show that Brahma, the ATPase of the SWI/SNF chromatin-remodeling complex, modulates the abundance of trans-spliced transcripts derived from the *mod(mdg4)* locus of *Drosophila melanogaster*. We have characterized the expression of anti-sense *mod(mdg4)* transcripts in S2 cells, mapped transcription start sites and cleavage sites, identified and quantified cis-spliced and trans-spliced transcripts, and got insight into the regulation of the *mod(mdg4)* trans-splicing. Using RNA interference and over-expression of recombinant Brahma proteins, we show that the levels of Brahma affect the levels of a trans-spliced *mod(mdg4)* mRNA isoform in S2 cells. The trans-splicing effect is independent of the ATPase activity of Brahma, which suggests that the mechanism by which Brahma modulates trans-splicing is independent of its chromatin remodeling activity. Interestingly, we also observed a similar effect on trans-splicing *in vivo* when reducing the levels of Brahma in larvae.

527-C THE RIBOSOME-OME II: ALTERNATIVE SPLICING FOR RIBOSOMAL PROTEINS?

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"Transcripts from ~95% of multiexon (human) *genes undergo alternative splicing."*[1]

"92-94% of human genes undergo alternative splicing. (sic)" [2]

Ribosomal proteins (RPs) are highly conserved across diverged species, a necessary their positioning in the compact, complex structure of the ribosome. To what degree does alternative splicing affect the nature and the structure of the ribosome? Could alternative splicing of transcripts of RP genes lead to production of proteins with non-ribosomal functions?

To approach these questions we have examined the splice junctions of RP gene transcripts from several multi-Gbyte RNA-seq databases. The results are as follows:

Abundant alternative splicing is rare. In no case did we observe tissue-dependent alternative splicing. In only one case, RPS24, have we observed substantial tissue-specific three-way alternative splicing, leading to S24 proteins whose C-termini: ···VGAGKKPKE vary in the last three amino acids.

There are many cases where one or even two exons are skipped, usually in less than 2% of observed transcripts. This usually leads to a truncated protein due to a nearby stop codon. Since this could result in nonsense-mediated decay, the actual frequency of such intron skipping could be much greater. For several genes, in a few % of cases an intron is mis-spliced (?), leading to the deletion of one to 19 aa from the middle of the RP. Are such RPs incorporated into the ribosome?

Finally there are several cases in which 0.1 to 5% of the mRNAs are mis-spliced (?) to encode a protein with the N-terminal portion of a RP followed by 10 to 100 additional AA. Since RPs are very abundant proteins, these fusion proteins have the potential to play as yet unknown, non-ribosomal functions in the cell. In none of these cases were the appropriate tryptic peptides observed in the Mass Spec database GPMDB.

Supported by NIGMS RO1 25532

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

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[2] Wang &···· & Burge, Nature **456**, 470-476, 2008

RNA-Protein Interactions

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 530 C – 578 C, 721 C
Location: Main Hallway & Sanada Foyer

530 C	**Site-Specific Intercalation of Doxorubicin Disrupts the Iron-Responsive Element RNA – Iron Regulatory Protein Interaction**
533 C	**In vivo dynamics of SR protein-RNA interactions**
536 C	**AURA 2.0: empowering post-transcriptional regulatory networks discovery**
539 C	**Structural and Dynamic Investigation on ETR-3 RRMs and their Interaction with AU-rich RNAs**
542 C	**Dead End, a protein counteracting miRNA-mediated repression of tumour suppressor genes, contains non-canonical RNA binding domains**
545 C	**Characterization of the potential role for RNA-binding protein FUS/TLS in DNA damage response: A quantitative proteomic approach**
548 C	**Predicting RNA-Protein Interactions: The Hunt for the Code of Recognition**
551 C	**Codon-usage effects and functional characterization of physicochemical complementarity between mRNA and cognate protein sequences**
554 C	**Repetitive RNA unwinding of a single RNA helicase A**
557 C	**RBP atlas: an exploration of interactions between mRNA and proteins and their impact on cardiomyocyte biology**
560 C	**RNA helicase function in yeast ribosome biogenesis**
563 C	**Mechanism of action of the CCCH zinc finger protein TbZC3H11 upon heat shock**
566 C	**Division of labor: separation of loading and unwinding units in an oligomer of the DEAD-box helicase Ded1p**
569 C	**Structure-specific RNase footprinting in multiple cell types reveals protein-binding sites throughout the human transcriptome**
572 C	**Human DIS3L2 exonuclease is involved in the processing of tRNA-derived small RNAs**
575 C	**Poly(A) binding protein 1 and formation of processing bodies in human**
578 C	**Evidence of direct complementary binding between mRNAs and cognate proteins**
721 C	**Functional and structural characterization of a thermostable RNA/DNA dependent RNA polymerase**

530-C Site-Specific Intercalation of Doxorubicin Disrupts the Iron-Responsive Element RNA – Iron Regulatory Protein Interaction

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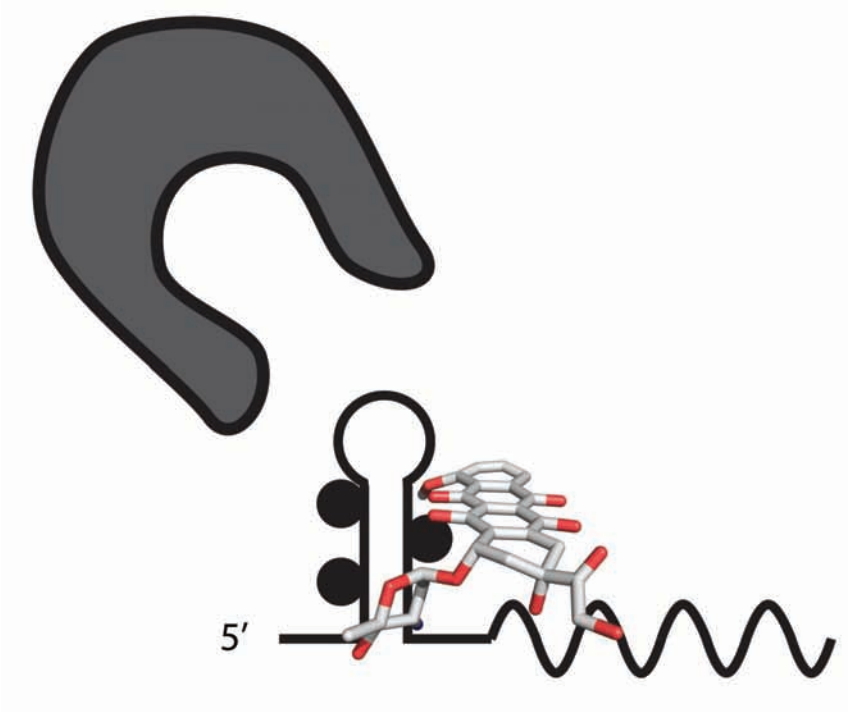
A widely utilized chemotherapy drug, doxorubicin, has recently been shown to bind to a mammalian 5' untranslated region Iron Responsive Element (IRE) RNA. In conjunction with the Iron Regulatory Protein (IRP), IRE RNA is involved in cellular iron homeostasis at the translational level. This tight RNA-protein complex prevents ribosomal assembly, hindering translation initiation of iron storage proteins, i.e. ferritin, under low cellular iron conditions. Conversely, iron overload is conducive to complex dissociation, allowing for up-regulation of the same proteins. However, this system is not entirely efficient. Some anemic patients receive adjuvant chelation therapies upon chronic blood transfusions to sequester excess labile iron. The use of doxorubicin to promote RNA-protein dissociation could potentially allow for downstream up-regulation of ferritin (see figure 1 below). In this work, we show how doxorubicin interacts specifically with IRE RNA using multidimensional nuclear magnetic resonance, fluorescence spectroscopy, and electrophoretic mobility shift assays. All three approaches converge on the observation that the IRE-IRP complex formation is disrupted by doxorubicin. Obtaining further data on the RNA-protein-drug interactions may lead to unveiling a validated RNA target as a complementary treatment of anemia.



533-C In vivo dynamics of SR protein-RNA interactions

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SR proteins are well known to play a key role as regulators of constitutive and alternative splicing. They show a modular structure characterized by one or two N-terminal copies of an RNA recognition motif (RRM) and a C-terminal serine/arginine (RS) dipeptide-rich domain. SR proteins act as splicing activators by binding ESE sequences via their RRM domain and then recruiting components of the splicing machinery. To select splice sites, SR proteins recognize short degenerated motifs present in multiple copies at ESEs. Similar cryptic motifs are also frequently present in pre-mRNAs and this low specificity of binding contrasts with the great fidelity splicing and exon definition. Our aim is to provide a detailed kinetic analysis of SR proteins-RNA interaction in living cells, by measuring the binding dynamics of SR proteins on model RNAs. To this aim, we use FRAP (fluorescence recovery after photobleaching) on GFP-SR tagged proteins and GFP-RRM tagged domains. To measure binding on specific RNAs, we FRAP the transcription site of MS2-tagged RNAs that contain repetitions of a binding site for an SR protein. In the long term, we aim at translating the statistical binding data obtained by techniques such as CLIP into a residency time on RNA, and we would like to test the hypothesis that combinatorial binding and protein-protein interactions can stabilize individual binding events.

536-C AURA 2.0: empowering post-transcriptional regulatory networks discovery

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The increasingly recognized importance of post-transcriptional regulation (PTR) is leading to the collection of more data than ever before. However, this pieces of data remain dispersed throughout many isolated databases or even lying in the literature, posing multiple obstacles to data integration and eventually preventing the discovery of the regulatory networks underlying these processes.

In order to address this issue, we originally developed the Atlas of UTR Regulatory Activity (AURA), now at its second and vastly enhanced iteration. AURA is a meta-database focused on all aspects of post-transcriptional regulation as mediated by the untranslated regions of mRNA. On top of a rich UTRs annotation layer, AURA contains experimental data on RNA-binding proteins and noncoding-RNAs binding sites, cis-elements, phylogenetic conservation and much more. Collected data covers multiple species, namely human and model organisms such as mouse, yeast and zebrafish. To allow for seamless data integration workflows, AURA also offers several data mining features, with particular focus on network generation, functional enrichment and UTR sequence analysis.

Regularly updated with the newest data and additional features, AURA aims at becoming a valuable toolbox for the PTR researcher and to stimulate the formation of an active community of users and contributors, able to eventually benefit the whole research field. AURA (soon available in its 2.0 version) is freely accessible at http://aura.science.unitn.it .

539-C Structural and Dynamic Investigation on ETR-3 RRMs and their Interaction with AU-rich RNAs

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The ELAV type RNA-binding protein 3 (ETR-3) is a RNA binding protein involved in many aspects of RNA metabolism. ETR-3 interacts with UG repeats in the context of splicing [1]. Sequence specificity for UG repeats and notably the UGUU motif has been confirmed by systematic evolution of ligands by exponential enrichment (SELEX) [2]. However, ETR-3 has also been shown to regulate the cycloxygenase-2 (COX-2) mRNA stability and translation by binding to AU-rich sequences in the 3'UTR region [3].

ETR-3 belongs to the CUG-BP and ELAV like factor (CELF) family. The members have two RNA recognition motifs (RRM) separated from a third one by a divergent domain (approx. 200 amino acids). The RRMs are extremely conserved among the family; over 90% identity between ETR-3 and CUG-BP1, its closest homolog. The solution structure of CUG-BP1 RRM3 in complex with $(UG)_3$ has been solved by NMR spectroscopy[4]and X-ray crystallography structures of CUG-BP1 RRM1 and RRM2 bound to RNAs containing the UGUU motif have been published [5]. Although these structures provide very detailed insight in the binding mode to UG rich RNAs, it remains unclear how ETR-3 recognizes AU rich RNAs and how both RRM1 and RRM2 are oriented upon RNA binding.

We aim at solving the structure of ETR-3 RRM12 and RRM3 bound to AU rich RNA. This will allow us to explain the discrepancies between the natural targets and the sequences obtained by SELEX. We established that RRM1 and RRM2 are semi-independent in the free form and we could show that both RRM12 and RRM3 bind to 5'-AUUUAAUU-3' sequence found in COX-2 mRNA. Dynamics studies on RRM12 in complex with the octamer demonstrated that RRM12 tumbles slower in complex than the free protein suggesting a rigidification of RRM12. In addition, we have a preliminary structure of RRM3 in complex with 5'-UUUAA-3'. In cell experiments demonstrated that RRM12 is sufficient to regulate the translation and unlike splicing, the divergent domain is not necessary for COX-2 mRNA translation inhibition.

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

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The protein Dead End (Dnd1) is necessary for proper development of germ cells in zebrafish and mammals [1]. Truncations in the Dnd1 gene have been shown to promote the formation of germ-cell tumors [2]. Recently the molecular function of Dnd1 has been exposed as a negative modulator of miRNA activity, when it was shown that it can counteract repression of several tumour suppressor genes (p27, LATS2) by the miR-221 family by blocking the accessibility of the mRNAs targeted by these miRNAs [3,4,5]. It was suggested that Dnd1 blocks access of miRNAs to their targets by binding to conserved U-rich regions (URRs) in close proximity to the miRNA seed sequences in the mRNA target 3'UTRs (see Figure 1 below).

Dnd1 contains two RNA recognition motifs (RRMs). To understand how Dnd1 recognizes its targets and inhibits miRNA-based gene silencing at the molecular level we aim to solve the three-dimensional structure of the Dnd1 RRMs in complex with RNA derived from one of Dnd1's 3'UTR-targets. We have identified short RNA oligomers derived from the p27 tumour suppressor mRNA 3'UTR that bind to the Dnd1 RRM1 and double RRM12 as shown by NMR chemical shift perturbation mapping and ITC. In addition, ITC measurements have shown that both RRMs are necessary for tight binding, suggesting that the two RRMs are working cooperatively in recognizing their mRNA-targets.

The solution structure of RRM12 shows that Dnd1's both RRMs contain non-canonical helical and beta-hairpin extensions to the classical RRM fold. The RNA binding site includes these non-canonical elements in addition to the RRM1 canonical beta-sheet RNA interaction surface. NMR based structure determination of a RRM12-p27-3'UTR complex is underway.

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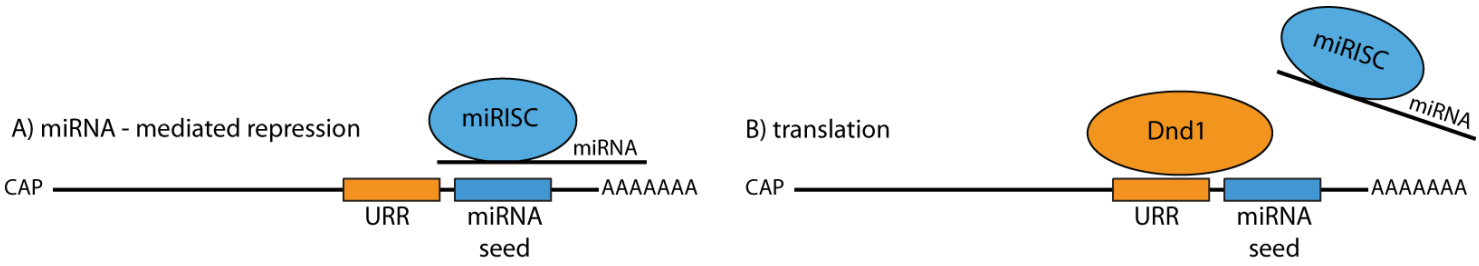

Figure 1:
A) miRISC binding through recognition of a complementary 'seed' sequence by the miRNA in the complex prevents translation of the mRNA
B) When Dnd1 binds to U-rich regions (URRs) next to the miRNA recognition sequence it blocks access of miRISC and the mRNA is translated

545-C Characterization of the potential role for RNA-binding protein FUS/TLS in DNA damage response: A quantitative proteomic approach

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FUS/TLS (fused in sarcoma/translocated in liposarcoma) is a ubiquitously expressed protein of the hnRNP family, that has been discovered as fused to transcription factors in several human sarcomas and found in protein aggregates in neurons of patients with an inherited form of Amyotrophic Lateral Sclerosis [Vance C. et al., 2009].

FUS is a 53 kDa nuclear protein that contains structural domains, such as a RNA Recognition Motif (RRM) and a zinc finger motif, that give to FUS the ability to bind to both RNA and DNA sequences. It has been implicated in a variety of cellular processes, such as pre-mRNA splicing, miRNA processing, gene expression control and transcriptional regulation [Fiesel FC. and Kahle PJ., 2011].

Moreover, some evidences link FUS to genome stability control and DNA damage response: mice lacking FUS are hypersensitive to ionizing radiation (IR) and show high levels of chromosome instability and, in response to double-strand breaks, FUS is phosphorylated by the protein kinase ATM [Kuroda M. et al., 2000; Hicks GG. et al., 2000; Gardiner M. et al., 2008].

Furthermore, preliminary results of mass spectrometric identification of FUS interacting proteins in HEK293 cells, expressing a recombinant flag-tagged FUS protein, highlighted the interactions with proteins involved in DNA damage response, such as DNA-PK, XRCC-5/-6, and ERCC-6, raising the possibilities that FUS is involved in this pathway, even though its role still needs to be clarified.

This study aims to investigate the biological roles of FUS in human cells and in particular the putative role in DNA damage response through the characterization of the proteomic profile of the neuroblastoma cell line SH-SY5Y upon FUS inducible depletion, by a quantitative proteomic approach. The SH-SY5Y cell line that will be used in this study expresses, in presence of tetracycline, a shRNA that targets FUS mRNA, leading to FUS protein depletion (SH-SY5Y FUS iKD cells). To quantify changes in proteins expression levels a SILAC strategy (*S*table *I*sotope *L*abeling by *A*mino acids in *C*ell culture) will be conducted on SH-SY5Y FUS iKD cells and a control SH-SY5Y cell line (that expresses a mock shRNA) and the relative changes in proteins levels will be evaluated after five and seven days upon FUS depletion, by nanoliquid chromatography coupled to tandem mass spectrometry (nLC-MS/MS) and bioinformatics analysis.

Preliminary experiments demonstrated that the SH-SY5Y FUS iKD cells, when subjected to genotoxic stress (high dose of IR), upon inducible depletion of FUS, showed a increased phosphorylation of *g*H2AX with respect to control cells, suggesting an higher activation of the DNA damage response.

548-C Predicting RNA-Protein Interactions: The Hunt for the Code of Recognition

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Protein-RNA interactions are found to be pivotal in a wide variety of cellular processes ranging from gene regulation to host defenses against pathogens. In addition to their fundamental biological regulatory role, the impact of faulty protein-RNA based regulation on metabolic and immunological diseases emphasizes the motivation for understanding the principles behind protein–RNA recognition for medical research. In the presented study, covariant residue patterns between families of interacting protein and RNA were investigated. Co-crystal structures of RNA binding proteins and RNA targets were collected from the Protein Data Bank and filtered in order to only retain crystals of non-synthetic molecules. The protein and RNA sequences that were found to interact using a five Angstrom distance threshold were extracted from the co-crystal structures and subjected to structural alignment against the Pfam and Rfam databases. Covariance analysis of the alignments of protein-RNA pairs were conducted using mutual information and the recently published RIsearch method [**1**] for RNA-RNA interactions was adapted to infer protein-RNA specificity models. This approach was verified on examples with well-known specificity and by comparison to interactions found in the co-crystal structures.

The presented study constitutes a method which can predict novel protein-RNA interactions, aid in the mapping of regulatory networks, and contributes to deciphering the code of recognition between protein and RNA.

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551-C Codon-usage effects and functional characterization of physicochemical complementarity between mRNA and cognate protein sequences

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We recently reported a persistent correlation between mRNA coding-sequence pyrimidine content and the propensity of cognate protein sequences to interact with pyrimidine mimetics as captured by the polar requirement amino-acid scale. Moreover, strong correlations were observed both on the whole proteome level and on the level of individual protein-mRNA sequence profiles. On the basis of these findings, we hypothesized that mRNA coding regions may in general be physically complementary to and therefore directly interact with cognate protein regions, especially if both polymers are unstructured. Here, we explore how codon usage affects these correlations by systematically varying the pyrimidine content of degenerate codons, while keeping the cognate protein polar requirement fixed. Next, we re-evaluate the correlations between the properties of two biopolymers, both on the level of sequence averages as well as individual profiles, and compare them against correlations observed for the native sets. We find that the levels of matching in present-day proteomes can be both significantly increased or decreased depending on particular codons, while still preserving the specific native codon usage bias for all proteomes tested (*M. jannaschii*, *E. coli*, *S. cerevisiae* and *H. sapiens*). Finally, we explore the relationship between the level of mRNA-protein complementary matching and biological function by analyzing the enrichment or depletion of functional Gene Ontology terms in different segments of the profile-matching distribution and find that certain molecular functions can easily be related to the mRNA-protein complementarity hypothesis.

554-C Repetitive RNA unwinding of a single RNA helicase A

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RNA helicase A (RHA) plays diverse roles in cellular machinery as a transcriptional co-activator, an essential cofactor for normal gastrulation during mammalian embryogenesis and a translational activator of complex cellular and retroviral mRNAs as like human immunodeficiency virus (HIV). Consisting of two double stranded RNA binding domains (dsRBDs) at the N-terminus, helicase core and a single stranded RNA (ssRNA)-binding RGG domain at the C-terminus, dsRBDs and RGG domain of RHA have been proposed to possess regulatory functions in its helicase activity. However, it has not been tackled yet how RHA plays a role as a transcriptional co-activator and how the subdomain as like dsRBDs controls the helicase activity of RHA owing to lack in the molecular detail of the RNA unwinding process by RHA. Here, we provided the detailed molecular mechanism of RNA unwinding by RHA, taking an advantage of a single-molecule technique, which unveiled the molecular details of the RNA unwinding process by RHA, including the characterization of every single step in the RNA unwinding process, the repetitive unwinding characteristic of RHA after its loading to dsRNA, and dsRBDs-controlled RHA loading or activation. Moreover, we directly showed that the repetitive RNA unwinding by RHA cleared the hydrogen bonds in dsRNA efficiently, making it easily access to the complementary ssRNA, which gives a hint how RHA acts as a transcriptional co-activator in a molecular point of view.

557-C RBP atlas: an exploration of interactions between mRNA and proteins and their impact on cardiomyocyte biology

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RNA-binding proteins (RBPs) control all aspects of RNA fate and defects in their function underlie a broad spectrum of human pathologies. Employing a combination of UV-crosslinking of proteins to RNA in living cells with identification of those co-purifying with poly(A)+ RNA by mass spectrometry, we recently identified 860 proteins as the "mRNA interactome" of human HeLa cells.[1] Over 300 of these RBPs were not previously known to bind RNA and their identification sheds new light on RBPs in disease, RNA-binding enzymes of intermediary metabolism, RNA-binding kinases, and RNA-binding architectures. We have now adapted this approach to identify the mRNA interactome of murine HL-1 cardiomyocytes. The HL-1 cell line maintains the ability to contract and other differentiated cardiac morphological and functional properties in culture.[2] Our analyses reveal both, RBPs commonly detected in cells of different origin as well as more cardiomyocyte-specific RBPs. Ongoing work is focused on detecting changes in the cardiomyocyte mRNA interactome under conditions of pathophysiological stress and on identifying the RNA targets of selected cardiomyocyte RBPs using RBP pull-down followed by next generation sequencing of co-purifying RNA. Altogether, these investigations will map networks of post-transcriptional gene regulation in cardiomyocytes and might reveal their involvement in molecular processes commonly at play in heart disease.

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560-C RNA helicase function in yeast ribosome biogenesis

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The synthesis of cytoplasmic ribosomes in Eukaryotes is best understood in the yeast *Saccharomyces cerevisiae*. Here, the pathway requires more than 200 non-ribosomal proteins, such as GTPases, nucleases and RNA helicases. In addition, 75 small nucleolar RNAs (snoRNAs) are involved and base-pair with pre-ribosomal RNA, and many are predicted to require helicase activities for their release. Since the knowledge on the 19 RNA helicases involved in ribosome biogenesis is still limited and molecular and regulatory functions of these enzymes seem to be diverse, the elucidation of their roles has remained a challenging task.

Using the UV cross-linking and analysis of cDNAs (CRAC) approach, Solexa deep sequencing and bioinformatics, we have identified RNA binding sites of helicases involved in ribosome biogenesis. One protein that we are studying is Rok1, a RNA helicase required for the maturation of the small ribosomal subunit. We have previously shown that Rok1 acts in the release of snR30, a box H/ACA snoRNA that is required for pre-rRNA processing. Interestingly, Rok1 crosslinks to a region in the eukaryotic expansion segment 6 of 18S rRNA at the snR30 basepairing site, which is in line with a direct involvement of Rok1 in the unwinding of snR30 from pre-ribosomal RNA.

We are currently analyzing CRAC data for several other RNA helicases, most of which are involved in the biogenesis of the large ribosomal subunit. These data will provide the basis for further functional analysis of the specific roles of RNA helicases in ribosome biogenesis.

563-C Mechanism of action of the CCCH zinc finger protein TbZC3H11 upon heat shock

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Most organisms induce transcription of heat-shock genes in response to temperature upshifts. However, Kinetoplastids, including important pathogens of human, animals and plants, control their gene expression almost entirely at the post-transcriptional level. Thus, amounts of heat-shock proteins after heat shock are regulated by mRNA stability and translation efficiency.

We showed previously that mRNAs encoding chaperones and co-chaperones are selectively stabilized by the *Trypanosoma brucei* CCCH zinc finger protein ZC3H11. Many target transcripts that interact with ZC3H11 contain a non-classical AU-rich element (UAU repeats) in the 3'-untransalted region. It has been shown that the ZC3H11 CCCH zinc finger domain is important for the interaction, with little discrimination between $(UAU)_n$ repeats and the classical AU-rich element which is bound by Tis-11 family proteins, $(UAUU)_n$ repeats.

Members of mammalian Tis11 family, however, contain two tandem CCCH zinc finger domains through which they bind to AU-rich elements, but in contrast, ZC3H11 has only one such domain. Hence, ZC3H11 should dimerize to gain reasonable RNA-binding specificity. Indeed, multimerization was apparent in gel-shift assays, and co-immunoprecipitation of V5- and myc-tagged versions of protein also confirmed dimerization.

It was shown that ZC3H11 expression is significantly induced upon heat shock. So, currently we are investigating the mechanism by which amounts of ZC3H11 and its activity are regulated. Like TTP and BRF-1, ZC3H11 is phosphorylated, but precise sites of the modification are as yet unknown. We do, however, have preliminary evidence that levels of ZC3H11 are – like those of TTP and BRF-1 – regulated through proteasomal degradation. Indeed, pulse-chase/immunoprecipitation analysis revealed that half-life of ZC3H11 protein increases upon heat-shock. In addition, polysome profiling showed that ZC3H11 mRNA is shifted to polysome fraction upon heat shock, what might explain elevated amounts of protein in response to temperature upshifts.

We propose that ZC3H11 operates via interactions with MKT1 and PBP1, which result in recruitment of PABP to the 3'-UTR and consequent RNA stabilization by protection from deadenylation.

566-C Division of labor: separation of loading and unwinding units in an oligomer of the DEAD-box helicase Ded1p

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DEAD-box RNA helicases perform ATP-dependent RNA and RNP remodeling reactions, including unwinding of RNA duplexes. In several DEAD-box proteins, RNA, nucleotide, and protein binding depend on the oligomeric state of the protein, but the impact of oligomerization on the function of DEAD-box helicases has not been examined. Here we have investigated oligomerization by the DEAD-box protein Ded1p from *Saccharomyces cerevisiae*.

During duplex unwinding, Ded1p forms an oligomer with at least three units. Two units of Ded1p associate with single stranded RNA proximal to duplex regions, and generate nearly all of the ATP hydrolysis observed during an unwinding reaction. Replacing the single stranded RNA with DNA, eliminates the majority of ATPase activity while having no significant effect on substrate binding or unwinding activity. Immobilization of two units of Ded1p on the single stranded RNA with a non-hydrolyzable ATP analog inhibits ATPase activity while promoting unwinding. Together these data suggest that two units of Ded1p serve as a loading platform to direct a third unit of Ded1p to the duplex for unwinding. We further show that the loading and unwinding units of the oligomer are functionally distinct in both ATP utilization and RNA binding, and we find that the C-terminal domain of Ded1p is critical for oligomerization. Moreover, a physiological interaction partner of Ded1p, eIF4G, interacts through the C-terminus of Ded1p and prevents oligomerization. Collectively, our findings reveal that oligomerization plays an important and underappreciated role in the biochemical function of Ded1p.

569-C Structure-specific RNase footprinting in multiple cell types reveals protein-binding sites throughout the human transcriptome

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RNAs are continuously associated with RNA-binding proteins (RBPs), and these interactions are necessary for their function and regulation. RBPs bind to target RNAs through sequence and/or structure-specific interactions. Increasingly, *in vivo* binding sites and sequence motifs for RBPs are being determined through the use of Crosslinking and Immunoprecipitation (CLIP) experiments, and several variant protocols. While these approaches have proven useful in determining the binding locations of individual RBPs, no current method allows global assessment of the extent and diversity of RNA-RBP interactions in both unprocessed and mature mRNAs. We developed a structure-specific RNase-mediated protein footprint sequencing approach to reveal the protein-protected sites (PPSs) of RNAs throughout the transcriptome, and applied it to three human cell types. The structure-specific nature of this assay allows for empirical determination of RNA base-pairing probabilities at protein-interaction sites. From this analysis, we uncover general principles of RNA-protein interactions, including an increased propensity for protein binding in the 3' UTR near the stop codon. Furthermore, we identify known and putative RNA-protein interaction sites and RBP-bound sequence motifs, and using RNA-affinity chromatography combined with mass spectrometry, we identified proteins that bind to known and novel sequence and structural motifs. Finally, we find that disease-associated single-nucleotide polymorphisms (SNPs) often affect RNA-protein interactions, revealing a likely molecular mechanism for numerous SNPs. Overall, we provide a global view of RNA-protein interaction sites and protein-bound RNA secondary structure in multiple cell types and provide a framework for the study of these features on a global scale.

572-C Human DIS3L2 exonuclease is involved in the processing of tRNA-derived small RNAs

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Processing of noncoding RNAs in eukaryotes involves the 3' to 5' exoribonucleolytic activity of type II ribonucleases that are part of the exosome complex. While in yeast it is known that the catalytic activity resides in Rrp44/Dis3 subunit, the functions of the three human Dis3 homologs have not been well characterized. In this work we aimed to uncover the physiological RNA targets of DIS3L2, the DIS3 homolog whose mutation is at the origin of the Perlman syndrome. We show that DIS3L2 is an exosome-independent cytoplasmic exoribonuclease, and through crosslinking and immunoprecipitation followed by RNA sequencing, we demonstrate that DIS3L2 is involved in the formation of tRNA-derived small RNAs (tRFs). A fraction of DIS3L2 along with its tRFs associates with ribosomes and DIS3L2 overexpression alters the polysome/monosome ratio, suggesting that DIS3L2 is involved in translational regulation. Most importantly, we found that the DIS3L2-dependent tRNA-derived fragments associate with Argonaute2 (AGO2) pointing to a crosstalk between the DIS3L2-dependent processing of tRNAs and AGO2-dependent regulation of gene expression the human cells.

575-C Poly(A) binding protein 1 and formation of processing bodies in human

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Poly(A) binding protein 1 (PABPC1) is an essential translation initiation factor. PABPC1 circularizes mRNA together with mRNA cap binding proteins, bridged by eIF4G. While PABPC1 is found at stress granules and absent from processing bodies in human, we find a mechanism how PABPC1 contributes to formation of processing bodies. This helps us understand the dynamics of RNA-protein granules.

578-C Evidence of direct complementary binding between mRNAs and cognate proteins

Bojan Zagrovic[1], *Mario Hlevnjak*[1], *Anton Polyansky*[1]

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The ability to interact with mRNA has recently been reported for many known RNA binding proteins, but surprisingly also for different proteins without recognizable RNA binding domains including several transcription factors and metabolic enzymes. In particular, direct binding to cognate mRNAs has been detected for various proteins creating a strong impetus to search for functional significance and basic physico-chemical principles behind such interactions. Here, we bioinformatically compare pyrimidine content of natural mRNA coding sequences with the propensity of cognate protein sequences to interact with pyrimidines (1). The latter is captured by polar requirement, an experimental measure of amino-acid solubility in aqueous solutions of pyrimidine mimetics pyridines. By analyzing proteomes of 15 different species, we find that pyrimidine density profiles of individual mRNAs remarkably mirror polar-requirement profiles of cognate protein sequences. For example, 4953 human proteins exhibit a correlation between the two with a Pearson coefficient $|R|>0.8$ (illustrated in Figure 1 for hemoglobin alpha-subunit, ATF3 and p53). In other words, pyrimidine-rich regions in mRNAs quantitatively correspond to regions in cognate proteins containing amino acids that prefer to interact with pyrimidine mimetics and vice versa. Moreover, by studying randomized genetic codes, we show that the natural genetic code is highly optimized to preserve these correlations. Finally, we derive interaction preferences between amino acids and RNA bases by analyzing known 3D-structures of protein-RNA complexes. Using this tool we both confirm the above findings for pyrimidines and also demonstrate an analogous behavior for purines with some exceptions. Overall, our results redefine the stereo-chemical hypothesis concerning the origin of the genetic code and provide evidence of direct templating of proteins from mRNAs before the development of ribosomal decoding. Moreover, our findings support the possibility of direct complementary interactions between mRNAs and cognate proteins even in present-day cells, especially if both are unstructured, with potential implications extending to all facets of nucleic acid/protein biology.

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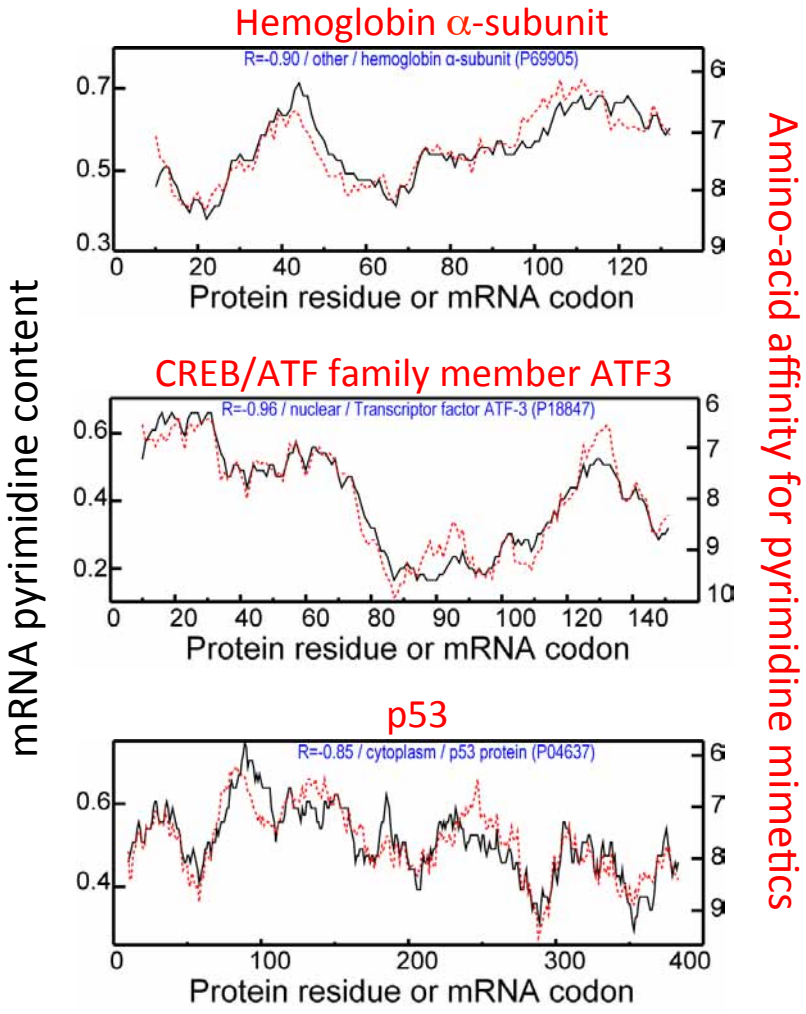

Figure 1 Legend

Examples of matching between mRNA coding-sequence pyrimidine-density profiles and cognate proteins' affinity profiles for pyrimidine mimetics. All profiles have been smoothed with a window of 21 codons/amino acids. Amino-acid affinity is defined in thermodynamic sense whereby low values indicate high affinity and vice versa.

721-C Functional and structural characterization of a thermostable RNA/DNA dependent RNA polymerase

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The discovery of RNA interference (RNAi) in the late 1990s has revolutionized biological research with great potential for therapy. Since double-stranded RNAs (dsRNAs) are required for various RNAi applications, there is a need for cost-effective methods for producing large quantities of high-quality dsRNA molecules, including using enzymatic approaches, as an alternative to chemical synthesis. Here, we characterize at the functional and structural level a thermostable RNA polymerase named QT2 that can use either DNA or RNA as a template. We present the crystal structure of the catalytic domain of QT2 (113 kDa) at a resolution of 3.2 Å. The results indicate that the enzyme could form a useful tool to generate large pools of dsRNA molecules. We will present our structural studies with a view to dissect the molecular basis of its various catalytic activities and the molecular basis for its thermostability.

RNP Structure, Function and Biosynthesis

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 581 C – 596 C
Location: Main Hallway & Sanada Foyer

581 C	**Characterization and in vivo functional analysis of the Schizosaccharomyces pombe ICln gene**
584 C	**Ribosomal protein clusters orchestrate the hierarchical construction of eukaryotic large ribosomal subunit structural domains in vivo**
587 C	**The structural organization of the box C/D sRNP**
590 C	**Messenger Ribonucleoprotein Assembly Requires the DEAD-box Protein Dbp2 and Enzymatic Modulation by Yra1**
593 C	**Surveillance of spliceosomal snRNP assembly in the cell nucleus**
596 C	**The 5S RNP couples ribosome production to p53 regulation**

581-C Characterization and in vivo functional analysis of the Schizosaccharomyces pombe ICln gene

Adrien Barbarossa[1], Henry Neel[1], Etienne Antoine[1], Thierry Gostan[1], Johann Soret[1], Remy Bordonne[1]

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Numerous studies indicate that assembly of the eukaryotic spliceosomal snRNPs is a multistep process following an ordered pathway. The methylosome and the SMN (Survival of Motor Neuron) complexes are essential players in early steps of this pathway. The methylosome, composed by pICln, WD45 and PRMT5 proteins, recruits Sm proteins via the pICln subunit and symmetrically dimethylates arginines within the C-tails of Sm proteins. The SMN complex further facilitates the loading of Sm proteins onto the snRNAs resulting in the formation of snRNP particles. In these early steps, pICln is an important regulator of snRNP assembly since it acts as an assembly chaperone while the SMN complex acts as a catalyst.

To expand our understanding of pICln and SMN functional relationships in vivo, we performed a genetic analysis of an uncharacterized S. pombe pICln homologue. Although not essential, the S. pombe ICln protein is important for optimal yeast cell growth. The human pICln gene complements the icln? slow growth phenotype demonstrating that the identified SpICln sequence represents the bona fide human homolog. Consistent with the role inferred for human pICln using in vitro experiments, we found that the SpICln protein is required for optimal production of the spliceosomal snRNPs and for efficient splicing in vivo. Genetic interaction approaches demonstrate furthermore that modulation of ICln activity is unable to compensate for defects induced by SMN mutations, and reciprocally. Using a genome-wide approach and RT-PCR validation tests, we show also that splicing is altered differentially in icln? cells. Our data are consistent with the emerging view that splice site selection and spliceosome kinetics are highly dependent on the concentration of core spliceosomal components.

584-C Ribosomal protein clusters orchestrate the hierarchical construction of eukaryotic large ribosomal subunit structural domains in vivo

Michael Gamalinda[1], *Uli Ohmayer*[2], *Jelena Jakovljevic*[1], *Beril Kumcuoglu*[1], *Philipp Milkereit*[2], *John Woolford, Jr.*[1]

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Ribosomes, the protein factories of the cell, are fundamental players that link genotypes with phenotypes. These nanomachines are made up of two subunits, each characterized by ribosomal proteins interspersed over a complex lattice of the ribosomal RNA (rRNA) core. Biogenesis of ribosomes in eukaryotes begins in the nucleolus with the transcription of precursor ribosomal RNA (pre-rRNA). This primary transcript is then modified, processed, folded, and assembled with ribosomal proteins, as nascent subunits traffic to the cytoplasm. In yeast, these events require more than 200 *trans*-acting biogenesis factors, which are thought to facilitate alternating cascades of rRNA folding and protein binding.

Assembly of small subunits is better understood relative to large subunits, both *in vitro* in bacteria and *in vivo* in eukaryotes. Construction of small ribosomal subunits proceeds via the formation of two stable intermediates: the body domain is first formed, followed by the creation of the head domain. In eukaryotes, assembly of these structural domains is also correlated with early and late pre-rRNA processing steps, respectively. In contrast, description of assembly intermediates for eukaryotic 60S subunits has largely been limited to cataloguing their protein composition, and understanding very late cytoplasmic steps in subunit maturation. Information on the timing of construction of assembly neighborhoods in pre-60S particles, and how this is coupled to protein binding and pre-rRNA maturation remain fragmentary. In this study, we began to fill this gap by systematically analyzing the roles of r-proteins in 60S subunit assembly *in vivo*.

We show that assembly of eukaryotic large ribosomal subunits proceeds via the formation of three sequential pre-ribosomal intermediates that correspond to the proper folding and construction of specific neighborhoods in the large subunit. The solvent-exposed interface of the large subunit is first stably assembled, followed by the domain surrounding the rim of the polypeptide exit tunnel, then finally, the central protuberance and the subunit interface bearing the catalytic center. We also discuss how formation of these structural neighborhoods is coupled with catalysis of pre-rRNA processing steps and nucleocytoplasmic export, by stable association of critical *trans*-acting biogenesis factors required for these events. This significantly contributes to our understanding of how the large ribosomal subunit and similarly complex ribonucleoproteins are put together in living cells.

587-C The structural organization of the box C/D sRNP

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The post-transcriptional modifications of ribonucleotides occur during the biosynthesis of the RNA in functionally important regions and are essential for the structure, folding, stability and functions of the host RNAs [1].

Our research is focused on the most common RNA modification, which is ribose 2'-OH methylation. RNA methylation in eukaryots and archaea is mediated by box C/D s(no)RNP complex. The archaeal sRNP complex is asymmetric and constituted by three core proteins assembled around small non-coding guide RNA. The gRNA contains two similar conserved motifs: box C/D and box C'/D'. The C box (RUGAUAG, being R purine) is close to the 5' end and the D box (CUGA) is near the 3' end. The related boxes C' and D' are in the internal region. The gRNA in sRNP forms base pairs with complementary target RNAs and selects the 5th nucleotide upstream from box D for methylation [2].

The complex assembly is initiated by L7Ae interaction with the K-turn and K-loop motifs of the box C/D guide RNA. Nop5 binds to the preformed L7Ae-RNA core subcomplex and facilitates binding of the Fibrillarin, which is a SAM-dependent methyltransferase [3].

Recently, the box C/D sRNP has been crystallized using a symmetric guide sRNA consisting of two separate strands, base-paired with the corresponding targets [4]. The structure suggests that the catalytically active complex is constituted by two copies of each protein assembled around one molecule of gRNA (~200 kDa) and is consistent with the classical mono-RNP model.

Another study demonstrates that the full box C/D sRNP complex is constituted by four copies of each protein and two copies of gRNA (di-RNP, ~ 400 kDa). A cross-methylation mechanism, during which the movement of Fibrillarin to the substrate-guide double strand facilitates release of one copy of L7Ae, was proposed [5].

Therefore, the assembly architecture and the functional regulation of this important enzyme remain to date contradictory.

Here we embark in the structural study of the box C/D sRNP complex from *Pyrococcus furiosus* in solution with a combination of NMR, small angle nuclear scattering (SANS) and restrained molecular modeling. Our study reveals a surprising mode of assembly of the complex in solution, which supports the di-RNP hypothesis, while showing a substantially different mode of RNA and proteins assembly with respect to that previously published [5]. Our structure explains the selectivity of the methylation and turn-over and furthermore provides a structural understanding for the asymmetry of the complex in all organisms.

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590-C Messenger Ribonucleoprotein Assembly Requires the DEAD-box Protein Dbp2 and Enzymatic Modulation by Yra1

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Eukaryotic gene expression involves numerous biochemical steps that are dependent on RNA structure and ribonucleoprotein (RNP) complex formation. DEAD-box RNA helicases are one class of enzymes that play fundamental roles in RNA and RNP structure in all aspects of RNA metabolism. However, the precise biological roles for the vast majority of these enzymes are not fully understood.

In an effort to define the biological roles of DEAD-box proteins, our laboratory recently provided evidence that the DEAD-box protein Dbp2 functions in nuclear gene expression steps in *Saccharomyces cerevisiae*. Moreover, our studies showed that Dbp2 is an enzymatically active ATPase *in vitro* that is required for both repression of aberrant initiation and proper transcription termination. Furthermore, we found that Dbp2 associates with transcriptionally active chromatin, suggesting that this enzyme functions in co-transcriptional mRNP assembly. We now provide evidence that *DBP2* interacts genetically and physically with the mRNA export factor Yra1. In addition, we find that Dbp2 is required for *in vivo* assembly of mRNA-binding proteins Yra1, Nab2 and Mex67 onto poly(A)+ RNA. Strikingly, to uncover the biochemical mechanism, we find that Dbp2 displays strong annealing activity at levels higher than any other published DEAD-box proteins to date. Yra1 controls this activity by inhibiting duplex unwinding without decreasing ATP hydrolysis activity. Here, we will present evidence that Dbp2 functions as an mRNA chaperone, constituting a previously unrecognized mechanism for co-transcriptional assembly of mRNPs in the nucleus.

593-C Surveillance of spliceosomal snRNP assembly in the cell nucleus

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Assembly of spliceosomal snRNPs and their incorporation into the spliceosome has been well described. Here, we provide evidence about a surveillance pathway that detects snRNP assembly intermediates and sequesters them in a nuclear structure called the Cajal body. We inhibited different stages of tri-snRNP assembly and in all cases observed retention of immature snRNP complexes in Cajal bodies. Next, we show that the protein SART3 is essential for targeting and retention of non-assembled snRNPs in Cajal bodies. We provide evidence that SART3 associates with U4 and U6 snRNPs as well as with coilin, a building protein of Cajal bodies. SART3 thus represents the factor that interacts with non-assembled snRNPs and anchors them in Cajal bodies. In addition, depletion of SART3 sensitizes cells to inhibition of snRNP formation, which provides a functional significance for surveillance pathway. Finally we show that Cajal bodies are inducible structures and inhibition of snRNP assembly stimulates formation of Cajal bodies in primary fibroblasts that normally lack Cajal bodies. Surprisingly, induced Cajal bodies accumulate only those snRNPs whose assembly pathway was perturbed, which provides the first evidence that individual metabolic pathways that concentrate in Cajal bodies are independent.

596-C The 5S RNP couples ribosome production to p53 regulation

Katherine Sloan[1], *Nicholas Watkins*[1]

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Ribosomopathies, including Diamond Blackfan anemia and Treacher Collins syndrome, are genetic diseases caused by defects in ribosome biogenesis. In several of these ribosomopathies, activation of the tumour suppressor p53 in response to impaired ribosome production plays an important pathogenic role. Paradoxically, many ribosomopathies are also associated with cancer predisposition. It is proposed that when ribosome biogenesis is blocked, two ribosomal proteins, RPL5 and RPL11, function to activate p53. RPL5 and RPL11, together with the 5S rRNA, form the 5S RNP, an essential sub-complex of the large ribosomal subunit (LSU). Both RPL5 and RPL11 bind to and inhibit HDM2, the E3 ubiquitin ligase that targets p53 for degradation. Conflicting reports suggest that RPL5 and RPL11 function either together or independently to repress HDM2. Furthermore, it is currently unclear whether the third component of the 5S RNP, the 5S rRNA, also plays a role in this process. Indeed, mechanistic insight into 5S RNP formation, its integration into the ribosome, and how this is coupled to p53 regulation is lacking in human cells.

Here we show that the 5S rRNA, as well as RPL5 and RPL11, is indeed required for p53 activation when ribosome biogenesis is blocked. In addition, the whole 5S RNP complex is required for p53 homeostasis in normal cells. We find that the levels of free, non-ribosomal 5S RNP in the nucleoplasm correlate with the amount of p53 in the cell. Indeed, our data indicate that factors that regulate 5S RNP formation, its nucleolar retention/localisation and its integration into the ribosome, determine the amount of free complex that can interact with and regulate HDM2 in the nucleoplasm. For example, knockdown of the human ribosome biogenesis factors RRS1 or BXDC1 inhibits 5S RNP nucleolar localisation and causes p53 activation. Furthermore, the known tumour suppressors, PICT1 and PAK1IP1, that both regulate p53, are in fact essential LSU biogenesis factors and PICT1 is needed for 5S RNP recruitment into the ribosome.

Following oncogene overexpression $p14^{ARF}$ inhibits ribosome biogenesis and activates p53 by repressing HDM2. We show that all three components of the 5S RNP are required for $p14^{ARF}$ mediated activation of p53. This provides evidence that blocking ribosome production is important for the cellular response to oncogene misregulation. Our data therefore reveal that the assembled 5S RNP complex, rather than its individual components, is a critical modulator of multiple signalling pathways coupling the regulation of cellular proliferation to ribosome biogenesis.

Riboregulation in Development

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 599 C, 719 C
Location: Main Hallway & Sanada Foyer

599 C **Cytoplasmic RNA regulatory networks orchestrate male gametogenesis**

719 C **Cold shock domain protein functions in reprogramming from differentiated cells to stem cells in the moss *Physcomitrella patens***

599-C Cytoplasmic RNA regulatory networks orchestrate male gametogenesis

Ryuji Minasaki[1], Christina Hirsch[1], Christian Eckmann[1]

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Tissue and organ formation requires the generation of complex protein expression patterns. Regulated protein synthesis, in the form of cytoplasmic post-transcriptional mRNA regulation, is a powerful mechanism to shape protein gradients across cells and tissues in space and time. Translational control is vital during metazoan germ cell development and early embryogenesis. Our past research was devoted to elucidating the molecular mechanisms of germ cell development in *Caenorhabditis elegans*. We primarily focused on the regulation of the tumor suppressor GLD-1, itself an RNA-binding protein and translational repressor. While GLD-1 protein expression is essential for female meiosis and *gld-1* mRNA regulation is a paradigm of translational control, loss of *gld-1* expression has no impact on male germ cell development. However, by studying the RNA regulatory network in males, we discovered essential and novel roles of highly conserved RNA-modifying enzymes and RNA-binding protein families.

A pivotal mechanism of translational control is anchored around the poly(A) tail, which influences mRNA stability and ribosomal engagement. Two opposing forces, deadenylases and non-canonical poly(A) polymerases, are envisioned to dynamically regulate poly(A) tail lengths in the cytoplasm, as a consequence of mRNA-associated factors, such as CPEBs. By focusing on male meiosis, we identified an intricate RNA regulatory web built of all four encoded CPEBs, which interface with two distinct cytoplasmic poly(A) polymerases, GLD-2 and GLD-4, and members of the PUF (Pumilio/FBF) protein family. Importantly, the process of meiotic chromosome segregation in most species is different between male and female germ cells. We find that these conserved sex-specific differences are reflected in a rewiring of the poly(A) tail control network in male vs. female germ cells to achieve gamete-specific gene expression. I will present our biochemical, genetic and cell biological data that reveal spatially and temporally resolved activities of all four CPEBs as master regulators of male germ cell development.

719-C Cold shock domain protein functions in reprogramming from differentiated cells to stem cells in the moss *Physcomitrella patens*

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Differentiated cells of the moss *Physcomitrella patens* have a high ability of being reprogrammed into stem cells. For instance, after leaf excision, differentiated leaf cells facing the cut are reprogrammed. Here we show that *P. patens* Cold Shock domain Protein (PpCSP), which is a homolog of one mammalian induced pluripotent stem cell (iPS) factor Lin28, plays a positive role in the reprogramming. In Physcomitrella genome, there are four *PpCSP* paralogous genes (PpCSP1 to PpCSP4), which encode highly similar RNA binding proteins. All of these four CSP proteins are localized in cytoplasm. The promoter activity and the localization of fluorescent proteins fused to the native PpCSP proteins showed that all PpCSP proteins accumulate in leaf cells facing the cut after excision and is maintained in reprogrammed stem cells. *PpCSP1* mRNA is regulated by putative degradation activity in its 3' UTR. Stabilized *PpCSP1* mRNA lines by excluding the degradation signal region showed enhanced reprogramming in cells not facing to the cut. Quadruple *PpCSP* mutants exhibited lower and delayed reprogramming. These data indicate that PpCSPs redundantly function as positive factors in the reprogramming. To reveal the molecular function of PpCSPs, transcriptome analysis was performed to seek downstream factors of PpCSP1. We found several candidate genes positively regulated by PpCSP1. Furthermore, to find the interacting factors of cytoplasmic PpCSP1 protein, we performed isobaric Tags for Relative and Absolute Quantitation (iTRAQ). PolyA-binding protein was identified and may have function together with PpCSP1. At last, by using ^{32}P labeled RNA, we confirmed that PpCSP1 could bind with mRNA in general. We currently search for target mRNAs of PpCSP1 by RNA-immunoprecipitation assay.

RNA Transport and Localization

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 602 C – 608 C
Location: Main Hallway & Sanada Foyer

602 C	**Insights into the nuclear mRNA export machinery of Trypanosoma brucei**
605 C	**Genome-wide identification of mRNAs associated with Survival of Motor Neuron proteins and whose axonal localization is decreased upon SMN deficiency**
608 C	**Ubiquitin and SUMO regulate Yra1: linking mRNA export factor to genome stability**

602-C Insights into the nuclear mRNA export machinery of Trypanosoma brucei

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Trypanosoma brucei is a unicellular protist parasite causing devastating diseases such as human sleeping sickness and Nagana in sub-Saharan Africa. Trypanosomes diverged early in evolution and show significant differences in their gene expression compared to higher eukaryotes. For instance, due to a lack of individual gene promoters, large polycistronic pre-mRNA molecules are transcribed from which individual mRNAs mature by *trans*-splicing and polyadenylation. In the absence of transcriptional control, regulation of gene expression occurs on the post-transcriptional level mainly by control of transcript stability and translation. We are investigating the process of nuclear mRNA export as a potential additional way of post-transcriptional gene regulation in *T. brucei*. The active translocation of mature mRNA from the nucleus to the cytoplasm is well understood in higher eukaryotes ranging from yeast to human - model organisms belonging to the eukaryotic supergroup of Opisthokonta. Their export factors such as the TREX complex and the export receptor Mex67-Mtr2 are well described and appear to be highly conserved. Trypanosomes are members of the supergroup of Excavata and the high divergence between Opisthokonta and Excavata makes it difficult to identify orthologous export factors based on sequence similarity. Hence, biochemical approaches are needed to identify the export machinery of the parasites. One of the few conserved factors are the orthologs of yeast Mex67 and Sub2. Here, we report the functional characterization of TbMex67. RNAi mediated downregulation of TbMex67 affects cell growth and leads to a nuclear retention of bulk mRNA. In contrast to all known orthologs, Mex67 of kinetoplastid species including *T. brucei* and other important pathogens like *T. cruzi* and *Leishmania* spp. contains a N-terminal zinc finger motif. We show that this parasite-specific feature is indispensable for the function of TbMex67. Overexpression of mutated versions of TbMex67 causes a dominant negative effect indicating that essential interacting proteins are sequestered. We used tandem affinity purification of PTP-tagged TbMex67 to identify such proteins and isolated two candidates that are essential for mRNA export as well. TbMtr2 interacts with TbMex67 to form the stable export receptor Mex67-Mtr2. TbIMP1 in contrast, belongs to the family of importins and is required for shuttling of TbMex67. Our data show that the heterodimeric export receptor is conserved throughout the eukaryotic kingdom. However, the zinc finger motif is unique to kinetoplastids and may play a parasite specific role.

605-C Genome-wide identification of mRNAs associated with Survival of Motor Neuron proteins and whose axonal localization is decreased upon SMN deficiency

Florence Rage[1], *Nawal Boulisfane*[1], *Rihan Khalil*[1], *Henry Neel*[1], *Thierry Gostan*[1], *Remy Bordonne*[1], *Johann Soret*[1]
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Spinal Muscular Atrophy is a neuromuscular disease resulting from mutations in the *SMN1* gene which encodes the Survival Motor Neuron (SMN) protein**.** SMN is part of a large complex that is essential for the biogenesis of spliceosomal small nuclear RNPs. Using SMA animal models, it has been reported that SMN deficiency, similar to that occurring in severe SMA, alters the stoichiometry of snRNAs and causes widespread pre-mRNA splicing defects in numerous transcripts of diverse genes (1, 2). However, whether the specific degeneration of motor neurons is caused by one or more aberrantly spliced transcript or by the cumulative effect of numerous splicing alterations remains to be determined. SMN also colocalizes with mRNAs in granules that are actively transported in neuronal processes and it was recently shown that SMN knockdown results in a reduction of poly(A) mRNA levels in the axonal compartment of primary motor neurons (3), suggesting thereby that SMN-containing complexes might be involved in the axonal localization of a large number of mRNAs. However, the full repertoire of SMN-associated RNAs has not yet been characterized.

To address this question, we have used murine motor neuron-like NSC-34 cells and RNA Immuno-Precipitation experiments coupled to microarray analyses (RIP-Chip) to perform a genome-wide analysis of RNA species present in mRNP complexes containing either the full length SMN protein (fl-SMN) or a recently described truncated axonal form (a-SMN). This approach allowed us to identify distinct but overlapping subsets of mRNAs associated with fl-SMN and a-SMN. Combination of fluorescent *in situ* hybridization (FISH) and immuno-fluorescence (IF) experiments indicated that several mRNAs colocalize with the SMN proteins in neurites and axons of differentiated NSC-34 cells. Interestingly, the axonal localization of some of these mRNAs is affected in SMN-depleted cells, suggesting that SMN-deficiency could result in the mislocalization of numerous mRNPs required for axonogenesis.

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608-C Ubiquitin and SUMO regulate Yra1: linking mRNA export factor to genome stability

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The nuclear periphery is a fundamental compartment to organize DNA domains and ensures proper transcription, replication and genome integrity.

The Nuclear Pore Complex has a well-established role in telomere tethering and subtelomeric repression at the nuclear periphery (Therizols, et al. 2006) and the loss of NPC subunits is synthetic lethal with components of the homologous recombination pathway (Pan X, et al. 2006). Intriguingly, the NPC physically interacts with the SUMO-dependent ubiquitin ligases Slx5-Slx8 that localize at irreparable DSBs (Nagai, et al. 2008). Moreover, the NPC-bound SUMO-protease Ulp1 prevents accumulation of DSBs (Palancade, et al. 2007) underlining the importance of sumoylation and ubiquitination in the DNA damage response.

Our earlier work (Iglesias, Tutucci et al. 2010) has shown that Yra1, an essential protein acting at the nuclear periphery and involved in transcription elongation, 3' processing, transcription termination and finally mRNA export, is released from the mRNP following ubiquitination by the E3 ligase Tom1 allowing proper mRNA export. Our recent studies identified Slx5-Slx8 as an additional E3 ligase involved in the regulation of Yra1 ubiquitination. Consistently, Yra1 is sumoylated and regulated by the protease Ulp1. Notably, the levels of Yra1 ubiquitination change in response to DNA damage. Our data highlight the importance of both sumoylation and ubiquitination in the regulation of Yra1 and suggest a new potential role of this mRNA export factor in the control of genome integrity.

RNAs in Diseases

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 611 C – 641 C
Location: Main Hallway & Sanada Foyer

611 C	**Altered microRNA expression profile in ALS: Role in the regulation of NFL mRNA levels**
614 C	**An Exon-Specific U1 approach to correct SMN protein deficiency in spinal muscular atrophy (SMA)**
617 C	**The RNA binding protein Quaking regulates monocyte adhesion and differentiation**
620 C	**hnRNP A2/B1 regulated alternative splicing of key signal transduction components and is essential for breast cancer metastasis.**
623 C	**Interplay of oncogenic transcription factor b-catenin with SR protein SRSF3 contributes to the alternative splicing of tumor-related genes**
626 C	**The Involvement of miRNA Dysregulation in Amyotrophic Lateral Sclerosis**
629 C	**Anti-inflammatory function of miR-146a in human primary keratinocytes and atopic dermatitis**
632 C	**Splice variant specific blood biomarkers of Parkinson's disease**
635 C	**Exonic splicing mutations in Mendelian disorders are more prevalent than currently estimated: the example of Lynch syndrome-associated MLH1 exon 10 variants**
638 C	**Spliceosome Integrity is Defective in the Motor Neuron Diseases ALS and SMA**
641 C	**RNA binding protein FUS acts to mediate nuclear-mitochondrial communication**

611-C Altered microRNA expression profile in ALS: Role in the regulation of NFL mRNA levels

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Amyotrophic lateral sclerosis (ALS) is a progressive, adult onset neurodegenerative disease of motor neurons (MN). The MN degeneration is associated with the formation of neurofilament aggregates and a selective suppression of low molecular weight neurofilament (NFL) mRNA. The preferential localization of NFL mRNA to P-bodies in ALS suggests that the suppression of its levels is related to an increase in RNA degradation. In addition, in vitro experiments in which human NFL (hNFL) mRNA was incubated with spinal cord (SC) homogenates pretreated with RNase showed that RNA species contribute to NFL mRNA destabilization in ALS. Considering that microRNAs (miRNAs) are key mRNA stability determinants and play a critical role in degeneration, they presented a likely candidate to explain our previous results. MiRNAs are small non-coding RNAs that participate in mRNA degradation mainly through base pairing interactions with the mRNA 3' untranslated region (UTR). In this study our objective was to characterize the miRNA expression profile in ventral lumbar SC tissue in sporadic ALS (sALS) and controls. We analyzed a large group of miRNAs and we found that the majority of dysregulated miRNAs are down-regulated in sALS. Ingenuity Pathway Analysis showed that these dysregulated miRNAs are linked with nervous system function and cell death. We used two prediction algorithms to develop a panel of miRNAs that have recognition elements within the hNFL 3'UTR. Finally, we investigated the functional relevance of these miRNAs using reporter gene assays and rqRT-PCR from HEK293T cells co-transfected with different hNFL mRNA 3'UTRs linked to a reporter gene and miRNAs predicted to interact with the 3'UTR. Our data suggest a potential role of several miRNAs in the selective decrease of NFL mRNA observed in ALS that could contribute to the etiology of neurofilamentous aggregates and the ALS pathology.

614-C An Exon-Specific U1 approach to correct SMN protein deficiency in spinal muscular atrophy (SMA)

Andrea Dal Mas[1], Erica Bussani[1], Malgorzata Rogalska[1], Franco Pagani[1]

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A significant proportion of disease-causing mutations affect pre-mRNA splicing inducing skipping of the exon from the mature transcript. We recently showed that modified U1snRNAs targeting non-conserved intronic sequences downstream of the 5'ss (Exon Specific U1s) are able to correct different types of splicing mutations associated with defective exon definition (Fernandez et al HMG 2012). Using coagulation *F9*, *CFTR* and *SMN2* models, we identified an ExSpeU1 able to rescue exon skipping caused by mutations at the polypyrimidine tract, the 5'ss or the ESEs. To investigate their efficacy and potential applicability *in vivo* we used a cellular model of spinal muscular atrophy (SMA) where improvement of SMN2 exon 7 skipping due to a silent exonic substitution is a reliable therapeutic strategy. In SMA-derived primary fibroblasts, lentiviral-mediated transduction of ExSpe U1s rescued SMN2 exon 7 splicing pattern. The increase in the amount of the correct full length (FL) mRNA resulted in a corresponding improvement of the SMN protein to a level present in unaffected fibroblasts. Furthermore, using the Hek293 Flip-In cell system, we show that corresponding levels of SMN2 exon 7 splicing rescue can be obtained by expression of only one chromosome-integrated copy of the ExSpe U1 gene.To understand the ExSpeU1s splicing enhancing mechanism we investigate the U1 components and the possible direct interference with ISSs. All model systems showed an ISS downstream the 5'ss. However, the ISS deletions rescued with variable efficiency only some types of splicing mutations and U7 RNAs targeting the ISSs had no effect on splicing suggesting that ExSpeU1 are only partially acting through an antisense mechanism that target the ISS.. On the other hand, ExSpeU1 mutants previously reported to affect 70K and U1A binding when tested in vivo, only slightly reduce, or do not affect, the splicing enhancement. In the CFTR case, side-directed mutagenesis showed that an RNA secondary structure-dependent accessibility of the 5'ss regulates splicing. All together, these data suggest that multiple factors are involved in the ExSpeU1-dependent improvement of exon definition

617-C The RNA binding protein Quaking regulates monocyte adhesion and differentiation

Ruben de Bruin[1], Janine van Gils[1], Hetty de Boer[1], Jacques Duijs[1], Erik Biessen[2], Ton Rabelink[1], Lily Shiue[3], Manuel Ares Jr[3], Anton Jan van Zonneveld[1], Eric van der Veer[1]

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Circulating monocytes are actively recruited to sites of tissue injury. The adherence of these cells to the activated endothelium leads to their extravasation into the sub-endothelial space where they differentiate into macrophages. Despite the fact that the cell surface receptors that regulate this adhesion process, such as p-selectin glycoprotein ligand-1 and b-integrins, have been characterized, the factors that regulate monocyte activation, adhesion and differentiation are poorly understood.

Here, we show that the RNA-binding protein Quaking (QKI) critically regulates monocyte activation, adhesion and differentiation. First, we identified that QKI is lowly expressed in human peripheral blood monocytes, while FACS sorting of monocyte subsets from peripheral blood and subsequent RNA isolation revealed that QKI mRNA expression is potently induced in a specific, activated subset of human circulating monocytes ($CD14^+$ $CD16^+$). Interestingly, the activation and differentiation of monocytes into macrophages was coupled with a striking induction of QKI protein (n=6). This finding was supported by laser capture micro dissection and immunohistochemistry studies where we validated that QKI mRNA and protein is highly expressed in macrophages in human atherosclerotic lesions. To gain mechanistic insight we utilized lentiviral short-hairpin RNAs to specifically abrogate QKI protein levels in human THP-1 and U937 monocytes. A reduction of QKI resulted in decreased adhesion using a cell perfusion assay ($p<0,05$ n=6), together with perturbed migration and differentiation. Subsequently, our discovery that QKI colocalizes with RNA species within spreading initiation centers of adhering monocytes, indicates that QKI could play a central role in regulating the translocation of RNA species required for the development of focal adhesions. Finally, we demonstrate that the transplantation of bone marrow from mice with decreased levels of QKI (quaking viable), into LDLR-/- mice fed a high-fat diet, leads to significantly reduced macrophage content in atherosclerotic lesions ($p<0,05$ n=13).

Collectively, we have identified that QKI critically regulates monocyte activation, adhesion and macrophage differentiation. We therefore propose that the targeted reduction of QKI in monocytes could be an effective means of limiting the severity of the systemic or local inflammatory response in disease.

620-C hnRNP A2/B1 regulated alternative splicing of key signal transduction components and is essential for breast cancer metastasis.

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Alternative splicing is a key control point in gene expression, and it is now becoming clear that it is also a process whose misregulation in cancer can contribute significantly to malignancy by regulating the expression of isoforms of various oncogenes and tumor suppressor genes. However, the role of alternative splicing regulators, or splicing factors, in cancer development and progression, is mostly unknown. Our recent studies indicate that some alternative splicing factors can be oncogenic, which partly involves the activation of known signaling pathways in novel ways.

Our results suggest that the splicing factor hnRNP A2/B1 is up-regulated in cancer and act as oncoproteins in several steps of cancer development and progression. Our aim is to use this factor as a model to reveal the functional roles of alternative splicing in cancer development, tumor maintenance and metastasis and the molecular mechanisms involved. Using a combination of both in vitro and in vivo cancer model systems in which we can manipulate the expression of hnRNP A2/B1 we discovered its role in cancer initiation and progression.

Since hnRNP A2/B1 is a broad regulator of alternative splicing, we hypothesize that its overexpression will change the splicing landscape of a large set of currently unknown target genes which might be novel determinants of survival, motility, invasiveness and other properties of tumor cells. In order to reveal the full spectrum of hnRNP A1/A2 splicing targets we performed RNA deep sequencing analysis of cells with hnRNP A2/B1 and discovered that it regulates several key pathways known to regulate invasion survival and metastasis.

This study discovered the biological functions of hnRNP A2/B1 in normal development and in cancer and identified the alternative splicing events it regulates. Furthermore, the therapeutic potential of our future discoveries is immense. Currently only a handful of drugable targets to treat cancer are available. The potential of identifying critical splicing events that are essential for cancer initiation or progression and can be modified by splice-site specific competitive oligonucleotides have great promise for future anti cancer therapy.

623-C Interplay of oncogenic transcription factor b-catenin with SR protein SRSF3 contributes to the alternative splicing of tumor-related genes

Jung Hur[1], *Sunjoo Jeong*[1]

[1]**Dankook University**

b-Catenin regulates the expression of many genes critical for cell proliferation and fate determination during development and stem cell maintenance under the control of Wnt signaling pathway. Constitutively active b-catenin was found in epithelial cancers including colon cancer, which indicates it as an important regulator for cancer initiation and progression. Interestingly, transcription factor b-catenin could also binds RNA, so it could influence altered transcriptome by regulating the splicing step of gene expression. Here we demonstrate that mutant b-catenin found in colon cancer cells significantly alters the splicing patterns of tumor-related genes, including p53 tumor suppressor gene. b-Catenin recognizes and associates near the alternative splicing site and regulates the alternative splicing. Interestingly, SRSF3 protein occupied the same alternative exon of p53 and repressed the alternative splicing, which could be abrogated by b-catenin. Such a crosstalk of the oncogenic transcription factor and splicing regulator could contribute globally to multitudes of genes important for tumorigenesis. We will discuss on a novel mechanism for b-catenin to regulate alternative splicing, and propose the model how b-catenin enhance the oncogenic RNA expression profiles in cancer cells. It will explain partially why b-catenin have a greater impact on oncogenic RNA expression network. Finally, we hope to provide new concepts in the therapeutic intervention of cancer development, targeting aberrant RNA binding property of b-catenin protein in cancer cells.

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626-C The Involvement of miRNA Dysregulation in Amyotrophic Lateral Sclerosis

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ALS is a neurodegenerative disease that specifically affects upper and lower motor neurons leading to progressive paralysis and death. There is currently no effective treatment. Thus, identification of the signaling pathways and cellular mediators of ALS remains a major challenge in the search for novel therapeutic approaches. Recent studies have shown that non-coding RNAs have a significant impact on normal CNS development and onset and progression of neurological disorders. Based on this evidence we specifically test the hypothesis that misregulation of miRNA expression is a common feature in familiar ALS. Hence, we are exploiting human neuroblastoma cell lines either expressing the SOD1(G93A) mutation or depleted from *Fused in Sarcoma* (FUS) as tools to investigate the role of miRNAs in familiar ALS. To this end we performed a genome-wide scale miRNA expression on these cells, using whole-genome small RNA deep-sequencing followed by quantitative real time validation (qPCR). This strategy allowed us to find a group of dysregulated miRNAs, which are predicted to play a role in the motorneurons physiology and pathology. We verified our data on cDNA derived from SOD1-ALS mice models at early stage of the disease and on cDNA derived from lymphocytes from a small group of ALS patients. In the future, we plan to define the mechanisms responsible for the miRNA dysregulation, by silencing or stimulating the signal transduction pathways putatively involved in miRNA expression and regulation.

629-C Anti-inflammatory function of miR-146a in human primary keratinocytes and atopic dermatitis

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Previously, miR-146a has been shown to regulate immune responses in different cell types. We carried out miRNA expression profiling and determined elevated expression of miR-146a in keratinocytes and skin from atopic dermatitis (AD) patients. Our results show that miRNA-146a is up-regulated by proinflammatory cytokines, such as TNF-alpha and IL1-beta, but not by IFN-gamma in primary keratinocytes. To study miR-146a functions further, we transfected miR-146a precursors or inhibitors into primary keratinocytes treated with IFN-gamma, TNF-alpha or IL1-beta. These experiments reveal that miR-146a hinders the capacity of primary keratinocytes to produce several inflammation-related cytokines and chemokines, such as CCL5, IL-8 and IL-6 both in mRNA and protein level. Over-expression of miR-146a suppresses proliferation and cytokine induced apoptosis of primary keratinocytes. mRNA array and pathway analysis of miR-146a-influenced genes demonstrates that miR-146a down-regulates mRNAs encoding proteins from NF-kappaB pathway as well as proteins involved in regulation of apoptosis and proliferation. siRNA inhibition of two miR-146a targets from NF-kappaB pathway, CARD10 and IRAK1 show that both these proteins are needed for production of IL-8, however, only CARD10 influences the expression of CCL5. We also demonstrate that miR-146a-deficient mice acquire similar Th2 type skin inflammation as wild type mice in mouse AD model. Concordant with the *in vitro* studies, elevated expression of IFN-gamma and CCL5 was detected in inflamed skin from miR-146a-/- mice, which indicates that anti-inflammatory function of miR-146a becomes more important in the chronic phase of skin inflammation. Together, our data show that miR-146a has a strong anti-inflammatory effect in human keratinocytes and its expression level may influence the course and intensity of AD-related skin inflammation.

632-C Splice variant specific blood biomarkers of Parkinson's disease

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Diagnosis of Parkinson's disease (PD) currently relies on assessment of motor symptoms. Distinguishing between PD and atypical parkinsonian disorders (APD) such as progressive supranuclear palsy and multiple system atrophy is sometime difficult based solely on clinical symptoms. Identification of sensitive minimally invasive risk markers would be beneficial so that proper therapeutic intervention may be initiated as early as possible. We used splice variant-specific microarrays to identify mRNAs whose expression is altered in peripheral blood of early-stage PD patients compared to healthy and neurodegenerative disease controls participating in the Diagnostic and Prognostic Biomarkers in Parkinson Disease study. Thirteen splice variants were identified that can be used to distinguish samples from PD patients and controls with 90% sensitivity and 94% specificity[1]. Six splice variants were identified that may be used to classify APD patients with 95% sensitivity and 94% specificity[1]. Preliminary results indicate that 9 of the risk markers may also be used to distinguish PD patients from healthy controls who participated in the Harvard NeuroDiscovery Biomarker study.

Network analysis of splice variant and transcription/RNA stability PD risk markers and genes associated with PD identified the transcription factor HNF4alpha (HNF4a) as a potential therapeutic target for PD. HNF4a plays a role in regulating genes involved in gluconeogenesis, lipid metabolism, and fatty acid metabolism. *Hnf4a* gene expression is up-regulated in the substantia nigra of PD patients[2] and the striatum of Parkinsonian mice[3].

[1]Potashkin et al., PLoS one 7: e43595

[2]Zhang et al., PloS one 5: e11464

[3]Kurz et a;., PloS one 5: e11464

635-C Exonic splicing mutations in Mendelian disorders are more prevalent than currently estimated: the example of Lynch syndrome-associated MLH1 exon 10 variants

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The identification of a causal mutation is essential for molecular diagnosis and clinical management of Mendelian diseases. Even if new-generation exome sequencing has greatly improved the detection of exonic changes, the biological interpretation of most exonic variants remains challenging. More often than not, the impact of exonic variants is evaluated by assuming a direct effect on protein sequence. However, it is currently known that exonic variants can also affect RNA maturation, stability and/or translation.

To evaluate the prevalence of RNA splicing mutations among disease-associated exonic variants we use *MLH1*, a gene implicated in hereditary colorectal cancer (also called Lynch Syndrome), as a model system. Here, we analyzed the effect on splicing of all single-substitutions reported on the Leiden Open Variation Database in the exon 10 of *MLH1* (n=22), including 15 missense, 3 nonsense and 4 synonymous mutations. *Ex vivo* splicing assays with representative minigenes revealed that 17 out of the 22 mutations had an impact on splicing. We detected the following alterations: (i) creation of an internal splice site, (ii) increased exon 10 inclusion, and (iii) increased exon 10 skipping. Remarkably, a significant number of variants located away from splice sites increased/decreased exon 10 inclusion (n=3/n=7), an effect that could not be predicted by commonly used bioinformatics approaches. These variants were further analyzed by using a completely heterologous minigene particularly sensitive to the presence of splicing regulatory sequences. Our results indicate that indeed these mutations alter exonic splicing regulatory elements. This observation led us to test a newly developed in silico tool aiming at detecting exonic splicing regulatory elements. With very few exceptions, we found the predictions produced by this new tool to be concordant with our minigene-derived data. When possible, results were also compared with those obtained by analyzing RNA extracted from patients' blood cells, and found to be concordant.

Overall, this study shows that the exon 10 of *MLH1* is very sensitive to splicing mutations, and indicates that the number of exonic splicing mutations in Lynch Syndrome, and very probably in many other Mendelian disorders, may be currently underestimated. Moreover, our results highlight the predictive potential of a newly developed in silico tool in pinpointing exonic variants that affect RNA splicing.

638-C Spliceosome Integrity is Defective in the Motor Neuron Diseases ALS and SMA

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Two motor neuron diseases, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), are caused by distinct genes involved in RNA metabolism, TDP-43 and FUS/TLS, and SMN, respectively. However, whether there is a shared defective mechanism in RNA metabolism common to these two diseases remains unclear. Here, we show that TDP-43 and FUS/TLS localize in nuclear Gems through an association with SMN, and that all three proteins function in spliceosome maintenance. We also show that in ALS, Gems are lost, U snRNA levels are up-regulated and spliceosomal U snRNPs abnormally and extensively accumulate in motor neuron nuclei, but not in the temporal lobe of FTLD with TDP-43 pathology. This aberrant accumulation of U snRNAs in ALS motor neurons is in direct contrast to SMA motor neurons, which show reduced amounts of U snRNAs, while both have defects in the spliceosome. These findings indicate that a profound loss of spliceosome integrity is a critical mechanism common to neurodegeneration in ALS and SMA, and may explain cell-type specific vulnerability of motor neurons (*Tsuiji et al., EMBO Molecular Medicine, in press*). We also found that TDP-43 lacking its Gly-rich region can regulate splicing of one of its target *Sortilin1*, but does not localize in Gem, does not associate with snRNPs, and shows reduced activity to down-regulate the expression of its own mRNA. These results indicate that the C-terminal Gly-rich region of TDP-43, in which most ALS-mutations reside, is important for full activity of TDP-43 and may play crucial role in neurodegeneration. To verify our hypothesis, we generated knock-in mice with TDP-43 lacking the Gly-rich region, and the expression of TDP-43 lacking the Gly-rich region in brain and spinal cord was confirmed. Further progress will be discussed.

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

Jane Wu[1]

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Genetic mutations in & dysregulation of the gene encoding Fused in Sarcoma (FUS) have been associated with FUS proteinopathies, neurodegenerative disorders including ALS and frontotemporal lobar degeneration with FUS pathology (FTLD-FUS). However, cellular defects underlying FUS proteinopathies are unclear. Our recent work show sthat FUS expression leads to increased fragmentation of mitochondria and production of reactive oxygen species (ROS). Immunoelectron microscopy (IEM) and biochemical fractionation experiments reveal that FUS is detected not only in the nucleus but also in association with mitochondria. Increased FUS protein levels accompanied by marked mitochondrial damage have been detected in brain samples of FUS proteinopathy. Screening genetic modifiers of FUS-induced neurodegeneration using transgenic flies expressing the human FUS led to identification of mitochondrial protein HSP60 whose downregulation partially rescued FUS-induced neurodegeneration. Our study uncovers a previous unknown role of FUS in mediating nuclear-mitochondrial communication. Our data provide evidence for mitochondrial damage induced by FUS expression and suggest that mitochondrial defects may be an early event in FUS proteinopathies. Slowing down or reversing mitochondrial damage may provide therapeutic benefits in treating these devastating diseases. Mechanisms underlying FUS-induced mitochondrial impairment and implications of our findings will be discussed.

Viral RNAs

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 644 C – 653 C
Location: Main Hallway & Sanada Foyer

644 C	**Deep-sequencing of the Peach Latent Mosaic Viroid Reveals New Aspects of Population Heterogeneity**
647 C	**Modulation of HIV-1 gene expression by binding of UHM-containing splicing factors to a ULM motif in the Rev protein**
650 C	**Domain interactions in adenovirus virus-associated RNA I mediate high-affinity PKR binding**
653 C	**RNA Packaging NTPase is Needed for Transcription in Double-stranded RNA Bacteriophage phi6**

644-C Deep-sequencing of the Peach Latent Mosaic Viroid Reveals New Aspects of Population Heterogeneity

François Bolduc[1], Jean-Pierre Sehi Glouzon[2], Rafael Najmanovich[1], Shengrui Wang[3], Jean-Pierre Perreault[1]

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Viroids are small circular single-stranded infectious RNAs that are characterized by a relatively high mutation level. Knowledge of their sequence heterogeneity remains largely elusive and previous studies, using Sanger sequencing, were based on a limited number of sequences. In an attempt to address sequence heterogeneity from a population dynamics perspective, a GF305-indicator peach tree was infected with a single variant of the *Avsunviroidae* family member *Peach latent mosaic viroid* (PLMVd). Six months post-inoculation, full-length circular conformers of PLMVd were isolated and deep-sequenced. We devised an original approach to the bioinformatics refinement of our sequence libraries involving important phenotypic data, based on the systematic analysis of hammerhead self-cleavage activity. Two distinct libraries yielded a total of 2,186 different PLMVd variants. Sequence variants exhibiting up to ~20% of mutations relative to the inoculated viroid were retrieved, clearly illustrating the high level of divergence dynamics within a unique population. Moreover, we show that ~50% of positions remained perfectly conserved, including several small streches as well as a small motif reminiscent of a GNRA tetraloop which are the result of various selective pressures. Using a novel hierarchical clustering algorithm, the different variants harvested were subdivided into either 7 or 8 clusters depending upon the library analyzed. We found that most sequences contained an average of 4.6 to 6.3 mutations compared to the variant used to initially inoculate the plant. This study provides a reliable pipeline for the treatment of viroid deep-sequencing. It also sheds new light on the extent of sequence variation a viroid population can sustain, and which may give rise to a quasi-species.

647-C Modulation of HIV-1 gene expression by binding of UHM-containing splicing factors to a ULM motif in the Rev protein

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Human immunodeficiency virus type 1 (HIV-1) is a lentivirus encoding the Gag, Pol and Env proteins common to all retroviruses and several specific regulatory proteins. The HIV-1 regulatory protein Rev is essential for virus replication and ensures the expression of partially spliced and unspliced transcripts. Rev binds to the Rev-responsive element (RRE) in viral mRNAs in the nucleus and recruits factors required for the export of these viral mRNAs to the cytoplasm. Numerous additional functions have been shown for Rev and Rev has also been implicated in regulating the splicing of viral transcripts.

We identified a ULM-like motif in the RRE-binding region of the Rev protein. ULMs (UHM ligand motifs) mediate protein-protein interactions and modulate spliceosome assembly through their binding to UHMs (U2AF homology motifs). We therefore investigated whether the Rev ULM can interact with UHMs present in host splicing factors. Isothermal titration calorimetry and NMR titration experiments showed that Rev ULM binds to the UHMs of SPF45 and U2AF65. The crystal structure of the SPF45-UHM bound to the Rev ULM revealed that the Rev ULM adopts an extended conformation upon binding to SFP45. Structural analysis and biochemical experiments demonstrate that the highly conserved W45 in the Rev ULM is crucial for Rev-UHM interactions. Moreover, W45 was shown to ensure the ability of SPF45 to displace single Rev subunits from Rev-RRE SLIIB oligomeric complexes. Finally, we show, that W45 is important for proper processing of HIV transcripts in human cells. We propose that W45 and thus Rev-ULM interactions with UHM splicing factors contribute to the regulation of HIV-1 splicing and gene expression.

650-C Domain interactions in adenovirus virus-associated RNA I mediate high-affinity PKR binding

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Adenovirus virus-associated RNA I (VA I) is a short, non-coding transcript that functions to inhibit the activity of protein kinase R (PKR), a component of the innate immunity pathway. PKR recognizes dsRNA and other RNAs containing duplex regions and is activated via dimerization and autophosphorylation. VA I contains three domains: an apical stem-loop, a highly-structured central domain, and a terminal stem. Although all three domains contain duplex regions that are of appropriate length for PKR recognition, previous work suggests that PKR binding is localized to the apical stem and central domain regions. We have characterized the stiochiometry and affinity of PKR binding to VA I and several deletion constructs using analytical ultracentrifugation. PKR binds to wild-type VA I with a 1:1 stoichiometry and a surprisingly high affinity (Kd = 334nM). Although PKR is capable of binding the isolated terminal stem, deletion of this domain does not affect PKR binding affinity or inhibition of autophorylation. Consistent with this observation, chemical probing data show that the secondary and tertiary structure of the apical stem and central domain do not change upon removal of the terminal stem. PKR is capable of binding to the isolated apical stem, albeit with greatly-reduced affinity, but does not bind to the isolated central domain. These results indicate that interactions between the apical stem and the central domain are necessary to form a high-affinity PKR binding site. Our data support a model whereby VA I functions as a PKR inhibitor because it binds a monomer tightly but does not permit PKR dimerization.

653-C RNA Packaging NTPase is Needed for Transcription in Double-stranded RNA Bacteriophage phi6

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Bacteriophage phi6 has three segments of genomic double-stranded RNA [S (2948bp), M (4063bp) and L (6374bp)] which are enclosed into a polymerase complex (PC), a particle displaying icosahedral symmetry. The PC of phi6 is a delicate enzymatic machinery which can selectively recognize and package the plus-sense single-stranded RNA (ssRNA) genomic precursors, preform minus-stranded synthesis to form dsRNA genomes (replication) and apply dsRNA as a template for plus-stranded synthesis (transcription). phi6 PC is composed of the main structural proteins P1, the RNA-dependent RNA polymerase P2, the packaging nucleoside triphosphatase (NTPase) P4 and the assembly cofactor P7. The hexameric packaging NTPase P4 resides on 5-fold symmetric vertex of the outer surface of PC. It is essential for the PC nucleation during self-assembly and required for phi6 ssRNA packaging and transcription. The particles with ~90% reduced level of P4, referred to P4-deficient particles, can only preform ssRNA packaging and replication but do not display the transcription activity. In this study, we applied the purified P4 on P4-deficient particles for assembly studies. The reconstituted PC products had high occupancy of P4 with a higher velocity in rate-zonal centrifugation than the P4-deficient particles. Further morphological analyses indicated that the P4 reconstitution induced conformation change of the shell from an expanded form to a compact form, which suggested that P4 occupancy level contributed to the dodecahedral symmetry of the empty PC. Our result also indicated that the transcription reaction was dependent on high occupancy of P4 hexamers on PC, which might be attributed to the access provided for export of nascent plus-sense ssRNAs.

Interconnections Between Gene Expression Processes

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 656 C – 671 C
Location: Main Hallway & Sanada Foyer

656 C	**Identification of RNA targets for the nuclear multidomain cyclophilin atCyp59 and their effect on PPIase activity**
659 C	**Not5 of the CCR4-NOT complex contributes to assembly of the SAGA coactivator complex.**
662 C	**Widespread regulation of mRNA steady-state levels through alternative splicing-dependent mechanisms**
665 C	**The Bre5-Ubp3 complex links RNA surveillance to RNA Polymerase II regulation by ubiquitylation**
668 C	**IMAGEtags for imaging Pol II activity in real time with RNA reporters**
671 C	**The human protein Nol12- ribosome biogenesis meets DNA damage and senescence**

656-C Identification of RNA targets for the nuclear multidomain cyclophilin atCyp59 and their effect on PPIase activity

Olga Bannikova[1], Marek Zywicki[2], Yamile Marquez[1], Tatsiana Skrahina[1], Maria Kalyna[1], Andrea Barta[1]

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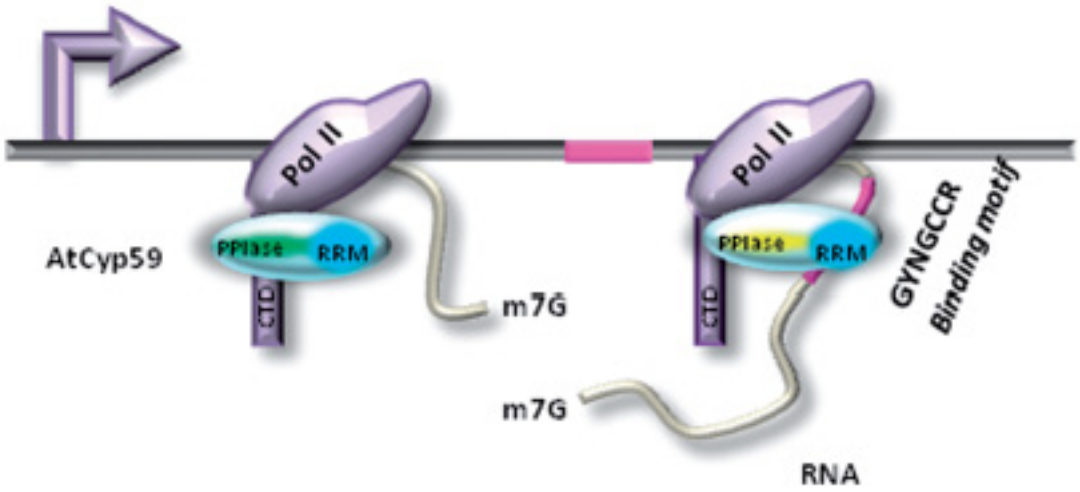
AtCyp59 is a multidomain cyclophilin containing a peptidyl-prolyl cis/trans isomerase (PPIase) domain and an evolutionarily highly conserved RRM domain. It has been shown to bind to the C-terminal repeat domain (CTD) of RNA polymerase II and to influence transcription significantly. To isolate RNA targets of AtCyp59 we employed a genomic SELEX method which is an unbiased approach to select potential RNA binding partners. Analysis of the selected RNAs revealed an RNA binding motif whose binding was verified by gel shift assays *in vitro* and by RNA immunopreciptation assays of AtCyp59 *in vivo*. Interestingly, genome-wide analysis showed that the consensus motif was present in at least 70 % of the annotated transcript and we show that this RNA motif is evolutionarily highly conserved. Most importantly, we show that binding also occurs on unprocessed transcripts *in vivo* and that binding of specific RNAs inhibits the PPIase activity of AtCyp59 *in vitro*. Taken together, the available data suggest that this type of cyclophilins have an important function in transcription regulation. Figure 1 presents a model where in the course of transcription RNA-dependent inhibition of the PPIase activity of AtCyp59 influences RNA polymerase II activity.

Figure 1



659-C Not5 of the CCR4-NOT complex contributes to assembly of the SAGA coactivator complex.

Sari Kassem[1], *Martine Collart*[2]

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CCR4-NOT is a conserved eukaryotic multi-subunit complex that plays a role at every step of gene regulation. A variety of evidences in our laboratory indicate that a unique function of the CCR4-NOT complex might be to contribute to the assembly of other multi-protein complexes. One such complex is the histone acetyltransferase and deubiquitinase SAGA complex. Former data have revealed that global histone acetylation is reduced in mutants of SAGA and CCR4-NOT, in particular in cells lacking the Not5 subunit. Our current results show that there is both physical and genetical interaction between the two complexes. In addition we find severe alterations in SAGA complex integrity in cells lacking Not5, which can explain the acetylation defect previously observed in cells, and which also correlates with several modifications of the core subunits of the SAGA complex. Further characterization of SAGA in wild-type and *not5* mutant cells has indicated that Not5 might connect a GAPDH family member, Tdh3, also known as a moonlighting protein, to SAGA, and thereby contribute to proper SAGA assembly. Taken together, our results suggest a key role for Not5 in both SAGA integrity and function.

662-C Widespread regulation of mRNA steady-state levels through alternative splicing-dependent mechanisms

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Differentiated cells acquire unique structural and functional traits through coordinated expression of lineage-specific genes. An extensive battery of genes encoding components of the synaptic transmission machinery and specialized cytoskeletal proteins is activated during neuronal differentiation, whereas genes required for neural stem cell (NSC) proliferation are turned off. We have recently shown that in non-neuronal cells, polypyrimidine tract-binding protein (Ptbp1/PTB/hnRNP I) represses splicing of 3'-terminal introns in pre-mRNAs encoding critical presynaptic proteins. This inhibits export of the incompletely spliced mRNAs to the cytoplasm and triggers their nuclear degradation. Clearance of these intron-containing transcripts occurs independently of nonsense-mediated decay (NMD) and requires components of the nuclear RNA quality control machinery. When Ptbp1 expression decreases during neuronal differentiation, the regulated introns are spliced out thus allowing translation-competent mRNAs to accumulate in the cytoplasm. Our unpublished work suggests that Ptbp1 additionally promotes expression of a large subset of non-neuronal genes by suppressing splicing patterns that lead to NMD. We show that this mechanism dampens the expression of these genes during neuronal differentiation. Thus, differentiation-induced changes in cellular mRNA steady-state levels appear to be frequently controlled by alternative splicing-mediated mechanisms.

665-C The Bre5-Ubp3 complex links RNA surveillance to RNA Polymerase II regulation by ubiquitylation

Laura Milligan[1], Sander Granneman[3], Grzegorz Kudla[2], Ross Alexander[1], Jean Beggs[1], David Tollervey[1]

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To identify novel nuclear RNA surveillance factors, yeast genome-wide screens were preformed for synthetic lethal (sl) interactions with loss of exosome cofactors Rrp47/Lrp1, Air1 or Air2. Each screen identified both Bre5 and Ubp3, which form a complex with protein de-ubiquitinase activity, suggesting links between RNA surveillance and ubiquitylation. Bre5 has a potential RRM and we confirmed RNA binding *in vitro* and *in vivo*. The CRAC UV crosslinking approach identified many Bre5 targets, transcriptome-wide. On intron containing genes, Bre5 hits were enriched over exon 2. Splicing-dependent transcription pausing over exon 2 has been reported, and a genetic screen using an allele of the splicing factor Prp45 that exacerbates this pausing also identified sl interactions with Bre5 and Ubp3. Ubp3 was previously shown to deubiquitylate RNAPII *in vitro* and levels of ubiquitylated RNAPII were elevated in *ubp3?* strains following DNA damage. CRAC was used to identify RNAPII interaction sites, transcriptome-wide; this provided data similar to ChIP, but strand specific and with nucleotide resolution. In addition, we specifically mapped the location of ubiquitylated RNAPII. This confirmed that levels of RNAPII ubiquitylation are elevated over Bre5 target genes. High-resolution, kinetic analysis of transcription and splicing of a reporter transcript revealed that the absence of Bre5 causes a delay in the release of RNAPII paused over exon II. We propose that splicing-induced, exon 2 pausing of RNAPII, results in its ubiquitylation to prevent further elongation while co-transcriptional splicing occurs. Recognition of the nascent transcript by Bre5 triggers de-ubiquitylation of RNAPII by Ubp3, allowing the polymerase to resume transcription following splicing. The Bre5-Ubp3 complex therefore provides a link between RNA surveillance and regulation of RNAPII activity by ubiquitylation.

668-C IMAGEtags for imaging Pol II activity in real time with RNA reporters

Judhajeet Ray[1], Ilchung Shin[1], Vinayak Gupta[1], Jonathan Beasley[2], Lee Bendickson[2], George A Kraus[2], Marit Nilsen-Hamilton[1]

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RNA based molecular imaging provides a new means of understanding of cellular events such as transcription and splicing. However the current techniques to visualize gene expression with RNA reporters are limited by the use of fluorescent proteins or by the need for highly abundant RNAs. We have developed an RNA reporter system known as IMAGEtags (Intracellular MultiAptamer GEnetic tags) for imaging promoter activity in real time in individual living cells by a FRET signal. The RNA reporter uses strings of RNA aptamers that can be expressed from an inducible or constitutive promoter. The tobramycin and PDC RNA aptamers were expressed in yeast from the GAL1, ADH1 or ACT1 promoters of a plasmid vector, which was transformed into the yeast cells. The cells are incubated with their ligands that are separately conjugated with the FRET pairs, Cy3 and Cy5. Following incubation with the dyes the cells are imaged. The constitutive ACT1 and ADH1 showed higher FRET signals compared to the control. FRET signals from the GAL1 promoter were seen to increase as a function of time after adding the galactose inducer and in parallel with the reporter RNA that was measured on a population basis. Cellular heterogeneity in FRET signals was also observed, which are consistent with the concept of stochasticity of transcriptional events in a cell population. IMAGEtags can be applied to studies of gene expression from pol II promoters in single cells and in real time. The system is simple, sensitive and applicable to many cell types and experimental conditions.

671-C The human protein Nol12- ribosome biogenesis meets DNA damage and senescence

Pierre-Joachim Zindy[1], Christian Trahan[1], Karen Wei[1], Jordi Ros Rodriguez[1], Marlene Oeffinger[2]

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It has been shown, in yeast as well as mammalian cells, that ribosome biogenesis and cell proliferation are two intimately linked processes. Defects in either one can lead to disease. To understand the differences between yeast and mammalian ribosome biogenesis and how the latter is integrated into cell division and proliferation pathways in higher eukaryotes, we are investigating the role of different ribosome biogenesis factors in human cells.

Here we focus on Nol12, the human ortholog of yeast Rrp17p, which has recently been described as a 5'-3' exonuclease required for 5' end processing of 5.8S and 25S rRNAs. Nol12 is a member of a conserved family of proteins and Nol12 orthologs have been related to dMyc-stimulated cell growth, proliferation and eye development in *Drosophila*, nucleolar architecture and function in rat as well as ribosome biogenesis in mouse.

We have studied the impact of Nol12 knockdown in human cells, using shRNAs, siRNAs, biochemical assays and proteomics. Cells lacking Nol12 are defective not only in pre-60S processing, as previously observed in yeast, but interestingly also in the maturation of 40S precursor RNAs. Moreover, we found that in the absence of Nol12, nucleolar architecture was severely affected, a hallmark for ribosome defects in mammalian cells. In addition, cells were not progressing through the cell cycle, accumulating in G1, a checkpoint that has been closely associated with defects in ribosome biogenesis; However, these cells were also unable to efficiently undergo G2/M progression, resulting in an accumulation of cyclin D1 and depletion of cyclin B1. In the past it has been shown that altered ribosome biogenesis is responsible for p53 stabilization through the action of several ribosomal proteins and MDM2, leading to G1 arrest, making this a p53-dependent response. However, in cells absent of Nol12, the effects on pre-rRNA processing, nucleolar architecture and G1 block seemed to occur in a p53-independent manner, while block of cells in G2/M was not observed in $HCT^{p53\ -/-}$ cells; c-myc was also not upregulated in these cells. Interestingly, a similar observation has been made in cells where disruption of 40S and 60S ribosome biogenesis was shown to lead to the activation of a novel G2/M checkpoint, while p53 was induced in these cells. In addition to cell cycle checkpoints, the DNA damage response pathway was also activated in the absence of Nol12 and cells went into senescence 48hrs after knockdown.

Our results suggest that the exonuclease Nol12 may have additional function to that during ribosome biogenesis, involving DNA damage, which in its absence leads to an activation of G1 checkpoint in a p53 as well as ribosome biogenesis-independent manner.

RNA system biology

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 674 C – 677 C
Location: Main Hallway & Sanada Foyer

674 C **Beyond the ribosome, antagonistic functions played by a pair of ribosomal proteins paralogs**

677 C **Large-scale analysis of eukaryotic RNA-binding protein binding preferences and exploration of their roles in post-transcriptional gene regulation**

674-C Beyond the ribosome, antagonistic functions played by a pair of ribosomal proteins paralogs

Anne-Cecile Duc[1], Yong Zhang[1], Shuyun Rao[1], Xiao-Li Sun[1], Alison Bilbee[1], Michele Rhodes[1], Qin Li[1], Dietmar Kappes[1], Jennifer Rhodes[1], David Wiest[1]

[1]Fox Chase Cancer Center

While ribosomal proteins are increasingly found to play a role beside their involvement in protein synthesis within the ribosome. It is also still controversial whether their paralogs have essential or distinct functions, and this has not been explored in vertebrates. Previous work from our lab showed that the Ribosomal Protein Large Subunit 22 (RPL22), or rather, its absence, interfered with T-cell development in a lineage-specific manner. Unlike the lack of most other ribosomal proteins that lead to deleterious effects, RPL22 deletion in mice specifically inhibits T-cell commitment to the a/ß, but not the G/? lineage. RPL22 paralog RPL22-Like1 does not rescue this lineage-specific defect. Strikingly, despite being highly homologous, RPL22 and RPL22-Like1 appear to play distinct and antagonistic roles. The focus of this research is to understand the roles of RPL22 and RPL22-Like1 in hematopoietic development. We found that they regulate the emergence of hematopoietic stem cells by controlling expression of Smad1. Emergence of T-cell lineage and hematopoeitic stem cells were investigated using zebrafish embryos, as well as *in vitro* RNA-proteins interactions to address this first example of ribosomal protein paralogs performing antagonistic functions in a tissue-restricted manner.

677-C Large-scale analysis of eukaryotic RNA-binding protein binding preferences and exploration of their roles in post-transcriptional gene regulation

Debashish Ray[1], Hilal Kazan[3], Kate Cook[5], Matthew Weirauch[2], Hamed Najafabadi[4], Xiao Li[5], Mihai Albu[1], Hong Zheng[5], Ally Yang[5], Hong Na[5], Serge Gueroussov[1], Manuel Irimia[1], Andrew Fraser[1], Benjamin Blencowe[1], Quaid Morris[1], Timothy Hughes[1]

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Hundreds of genes in eukaryotic genomes encode RNA-binding proteins (RBPs) but few have well-defined RNA-binding preferences or experimentally defined RNA targets. We have developed a combined biochemical and computational approach, termed RNAcompete, to analyze the RNA-binding preferences of RBPs (Ray, Kazan *et al.* Nature Biotechnology 2009). Motifs identified using RNAcompete haven been shown to be comparable to RNA-binding data from *in vitro* selection (e.g. SELEX) and immunoprecipitation (e.g. CLIP) experiments. Here, we use RNAcompete to conduct the first large-scale analysis of RNA-binding preferences for 207 RBPs from 20 diverse kingdoms across eukarya. We find that most RBPs do not have strict requirements for RNA secondary structure and bind to short ssRNA sequences. Furthermore, the binding motifs of homologous RBPs display deep evolutionary conservation, such that the recognition preferences for a large fraction of metazoan RBPs can be inferred from the sequences of their binding domains. We are also able to utilize RNAcompete-derived motifs during transcriptome analyses in metazoans to facilitate identification of RBP-RNA interactions enriched in mRNA subsets and associated with specific post-transcriptional processes. The collection of motifs derived from our RNAcompete experiments will be invaluable for understanding the interactions between RBPs and RNA, as well as their functional relevance.

Bioinformatics

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 680 C – 695 C
Location: Main Hallway & Sanada Foyer

680 C	**RNAs in silico: learning from accelerated molecular dynamics**
683 C	**Determining optimal flanking regions of RNA secondary structures for experimental analysis**
686 C	**Uncovering markers of cell identity change from transcriptome profiles**
689 C	**THE CONSERVED STRUCTURES OF RIGHT-HANDED POLYMERASES**
692 C	**Understanding without reading: analog encoding of physico-chemical properties of proteins in cognate messenger RNA**
695 C	**Searching the coding region for microRNA targets**

680-C RNAs in silico: learning from accelerated molecular dynamics

Giovanni Bussi[1]

[1]Scuola Internazionale Superiore di Studi Avanzati

Ribonucleic acid (RNA) is acquiring a large importance in cell biology, as more functions that it accomplishes are discovered. However, experimental characterization of RNAs dynamical behavior at atomistic level is difficult. Molecular simulations at atomistic detail, in combination with state-of-the-art free-energy techniques, can bridge the gap providing an unparalleled perspective on the mechanism and dynamics of RNA folding, conformational transitions, and of RNA/protein interactions. Two recent applications of these techniques will be discussed. The first is focused on a characterization of the zipping and unzipping mechanisms for a RNA double strand [1]. Results are compared with experimental findings, including analysis of X-ray data [2], ultrafast spectroscopy [3] and thermodynamic data [4]. Implications on the directionality of RNA processing enzymes are also discussed. The second application is a study of the interaction between TAR RNA from HIV and a cyclic binding peptide of pharmaceutical relevance [5]. This is done by introducing a suitable acceleration technique that allows for a blind prediction of the bound structure. Results are in nice agreement with previous NMR experiments [6].

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683-C Determining optimal flanking regions of RNA secondary structures for experimental analysis

Nikolai Hecker[1], Stefan E. Seemann[1], Jakob Hull Havgaard[1], Radhakrishnan Sabarinathan[1], Henrik Nielsen[4], Mikkel Christensen-Dalsgaard[4], Peter F. Stadler[3], Ivo Hofacker[2], Jan Gorodkin[1]

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Multiple sequence and structural alignments are often used to perform more accurate RNA secondary structure predictions than folding single sequences. As such they provide useful consensus structures for different experiments, e.g. structure probing. However, design of probing or other experimental analysis often require considering the structure of a single sequence in question and in addition a sequence flanking the predicted structure. Both aspects contribute to the structure of a single sequence to deviate from the corresponding consensus structure extracted from the structural alignment that contains the comparative information. To address this, we have developed a tool, RNAcfold (RNA context folding), that determines optimal flanking regions and can cope with arbitrary requirements for the minimum size of flanking regions up- and downstream of the specific structure in question. This is done by making use of constrained folding as implemented in the ViennaRNA Package while selecting the size of the flanking regions so that the probability for observing the structure in the region corresponding to the extracted structure from the structural alignment is maximized. In more detail, the probability is determined by the fraction of the partition function over all allowed secondary structures which satisfy the constraints compared to the partition function over all allowed secondary structures without constraints.

The Rfam database 11.0 was processed for number of sequences and structure content resulting in 324 suitable structure alignments. Next, the similarity between consensus structures and minimum free energy structures corresponding to optimal flanking regions or to fixed sized flanking regions was evaluated for each sequence. We evaluated the agreement between the folded structures (with and without optimal extension of flanking regions, respectively) to the consensus structure from Rfam using Matthews correlation coefficients (MCC). We report improvements for all combinations of minimum flanking regions in the range 0 to 50 (in steps of 10) for 5' and 3' UTRs. For example, a typical set up of a minimum 0 nt in 5'UTR and 30nt 3'UTR, the average improvement is 0.05 in MCC and for 15.74% of the families an improvement of more than 0.1 in MCC is obtained.

686-C Uncovering markers of cell identity change from transcriptome profiles

Nancy Mah[1], *Jean-Fred Fontaine*[1], *Miguel Andrade-Navarro*[1]

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Understanding the control of cellular reprogramming is crucial to improve and create protocols for its induction, to find out about the mechanisms that lead to pathological or failed changes of cell identity in cancer and developmental diseases, and to better understand cell pluripotency and differentiation. There is increasing evidence that modifications in common regulatory networks are required to facilitate changes of cell identity such as induced reprogramming, cancer and differentiation. For example, genes related to the epithelial to mesenchymal transition (EMT) are modified both during reprogramming and cancer. Our goal is to find genes expressed in cells that are undergoing changes in identity through the examination of gene expression profiles from cancer, reprogramming and differentiation.

689-C THE CONSERVED STRUCTURES OF RIGHT-HANDED POLYMERASES

Heli Mönttinen[1], *Janne Ravantti*[2], *Minna Poranen*[1]

[1]Department of Biosciences, University of Helsinki; [2]Department of Biosciences and Institute of Biotechnology, University of Helsinki

Viral RNA-dependent RNA polymerases (vRdRp) have the classical right-handed polymerase structure containing three conserved subdomains: thumb, palm and fingers. The catalysis reaction is run by two catalytic ions in the palm subdomain. Interestingly, in the vRdRp of the phage φ6, a manganese ion has been observed around 6 Å from the catalytic ion binding site. This non-catalytic ion was later shown to be needed for the template binding, nucleotide coordination and catalysis. An ion in a similar position has also been observed in several other vRdRps. Now, we have identified the non-catalytic ion binding site by using structural alignment. Our results suggest that the non-catalytic ion binding site is common for positive-stranded and double-stranded RNA polymerases. Furthermore, we have studied the common structural features and evolutionary relationships of the right-handed polymerases by using a novel structural classification method. This study covers vRdRps, single-subunit DNA-dependent RNA polymerases, RNA-dependent DNA polymerases and DNA-dependent DNA polymerases. These results provide information about the structural and functional conservation of the right-handed polymerases.

692-C Understanding without reading: analog encoding of physico-chemical properties of proteins in cognate messenger RNA

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Being related by the genetic code, messenger RNAs (mRNAs) and cognate proteins are polymers with mutually interdependent compositions, which further imply the possibility of a potential connection between their physico-chemical properties. How efficiently do different characteristics of mRNA coding regions reflect the features of cognate proteins and is it possible for the cell to obtain information about proteins from their mRNAs without first reading them on the ribosome? We address these issues in a theoretical proteome-wide analysis and show that average protein hydrophobicity, calculated from either sequences or 3D structures, can be encoded in an analog way by many different mRNA sequence properties with the only constraint being that pyrimidine and purine bases should be clearly distinguishable. Moreover, average characteristics of mRNA sequences allow for a reasonable discrimination between human proteins with different cellular localization and, in particular, cytosolic and membrane proteins, even in the absence of signal-peptide-based mechanisms. We discuss our findings in the context of protein and mRNA localization and propose that this cellular process may be partly determined by basic physico-chemical rationales and interdependencies between the two biomolecules.

695-C Searching the coding region for microRNA targets

Jiri Vanicek[1], *Ray Marin*[1], *Miroslav Sulc*[1]

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Finding microRNA targets in the coding region is difficult due to the overwhelming signal encoding the amino acid sequence. Here we introduce an algorithm (called PACCMIT-CDS [1]) that finds potential microRNA targets within coding sequences by searching for conserved motifs that are complementary to the microRNA seed region and also overrepresented in comparison with a background model preserving both codon usage and amino acid sequence. Precision and sensitivity of PACCMIT-CDS are evaluated using PAR-CLIP and proteomics datasets. Thanks to the properly constructed background, the new algorithm achieves a lower rate of false positives and better ranking of predictions than do currently available algorithms, designed to find microRNA targets within 3'UTRs.

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Emerging & High-throughput Techniques for RNA

Date: Saturday, June 15, 14:00 - 17:00
Abstracts: 698 C – 716 C
Location: Main Hallway & Sanada Foyer

698 C	**Full-Length Transcript Sequencing: Looking Beyond the ENCODE data**
701 C	**miR-Direct: RT-qPCR analysis of plasma microRNAs without prior RNA extraction**
704 C	**Post-transcriptional regulation of mitochondrial gene expression**
707 C	**Discovery of novel small molecules that bind HCV IRES RNA by SHAPE-directed screening**
710 C	**Screening of the RNase-sensitive subnuclear structures identified the Sam68 nuclear body that was built on RNA with novel protein components**
713 C	**Imaging trinucleotide repeat RNA in live cells using Spinach2, an RNA tag with enhanced brightness and thermostability**
716 C	**Identification of novel post-transcriptional regulatory sequences**

698-C Full-Length Transcript Sequencing: Looking Beyond the ENCODE data

Kin Fai Au[1], Elizabeth Tseng[3], Vittorio Sebastiano[4], Lawrence Lee[2], Renee Reijo Pera[4], Wing Hung Wong[1], Jason G. Underwood[3]
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Most RNA-seq experiments to date use short cDNA reads to assemble and count putative transcripts. These methods can infer alternative events such as splicing or transcription start sites, but cannot correlate events that are far apart on the original transcript since short reads cannot truly assemble the original, single RNA molecule. To address this deficiency, we applied the long read length capabilities of the PacBio® *RS* to sequence full-length cDNA molecules derived from human H1 stem cell polyA RNA. This cell line, a Tier 1 sample for the ENCODE project, has been extensively characterized by multiple 2nd generation sequencing approaches, including RNA-seq to assist in transcript assembly. We hypothesized that the long reads would help not only to assign the most common full-length mRNA isoforms in the H1 line, but also assist in assembling transcripts that are difficult with current algorithms.

We demonstrate that several full-length library preparation methods generate sequencing libraries that are highly enriched in full-length cDNA molecules. This was shown using a reference-based approach of aligning putative full-length cDNA reads to known transcripts in the Gencode set. By this alignment method, we detect many known full-length transcripts spanning a range from 500 bp to 6000 bp in length. We also present genome-based alignment approaches using gapped alignment methods that account for splicing events. To assist the genome-based approach, we have developed an error correction method, LSC, which uses short-read data to improve the alignments of long full-length cDNA sequences. Using both long and short reads, we developed an isoform detection and prediction pipeline. We report our findings on detecting novel splicing events and the discovery of new ncRNAs that are not apparent from Cufflinks short-read assembly alone. As an unintended benefit, this methodology also captures transcript maturation in action, as retained intron events can often be seen in the full-length reads. The methods and algorithms for long-read cDNA sequence determination will assist researchers to better characterize the transcriptome's true form and help unlock combinatorial RNA processing regulation that cannot be observed in previous RNA-seq data sets.

701-C miR-Direct: RT-qPCR analysis of plasma microRNAs without prior RNA extraction

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Circulating microRNAs (miRNAs) have great potential as biomarkers, but current methods for their detection are hampered by inconsistent RNA recovery during isolation, by the difficulty of eliminating RT-PCR inhibitors, and by the low concentration at which most miRNAs appear in blood. Here we describe a novel method (miR-Direct™) in which miRNAs or other small RNAs (or fragments of large RNAs) of interest can be enriched from plasma samples and directly quantified by RT-qPCR without prior extraction of total RNA. miR-Direct consists of the following steps: (1) treatment of plasma with agents that release miRNAs from lipid and/or protein complexes; (2) capture of miRNAs of interest by hybridization with specific probes attached to magnetic beads; (3) washing the captured miRNAs to remove inhibitors of amplification reactions present in plasma and the release buffer; (4) release of the captured miRNAs into solution; and (5) detection of the released miRNAs using an RT-qPCR assay. We compared two such assays, SomaGenics' miR-ID® and Life Technologies' TaqMan® microRNA assay, for detection of three circulating miRNAs (hsa-miR-16, hsa-miR-148a and hsa-miR-125b) as well as a spike-in control (cel-miR-39). In this comparison, miR-ID detection produced significantly lower cycle threshold (Ct) values than TaqMan detection, and Ct values showed the expected dependence on starting plasma volume only with miR-ID. The miR-Direct method reduces the variable loss of small RNAs that is usually seen with standard total RNA isolation conditions. Because sample processing (up to the qPCR step) is a one-tube process, the method is highly amenable to automation. Because irrelevant RNA and DNA is removed prior to the amplification steps, background is low and sensitivity high, resulting in accurate expression profiling of small RNAs and determinations of absolute copy numbers.

704-C Post-transcriptional regulation of mitochondrial gene expression

Aleksandra Filipovska[1], Ganqiang Liu[4], Stefan Siira[1], Timothy Mercer[3], Anne-Marie Shearwood[1], Dimitar Azmanov[1], Stefan Davies[1], John Mattick[2], Oliver Rackham[1]

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Human mitochondria contain a small and compact genome that is transcribed as long polycistronic transcripts that encompass each strand of the genome, which are processed into mature mRNAs, tRNAs and rRNAs within the mitochondrial matrix. Recently we provided the first comprehensive map of the human mitochondrial transcriptome by near-exhaustive deep sequencing of long and small RNA fractions from purified mitochondria (1). We have identified previously undescribed transcripts, including small RNA and long non-coding RNAs encoded by the mitochondrial genome (2). Furthermore despite their common polycistronic origin, we observed wide variation between individual tRNAs, mRNAs, and rRNA amounts, indicating the importance of RNA-binding proteins in the regulation of mitochondrial gene expression (3). We have investigated the roles of the mammalian pentatricopeptide repeat (PPR) proteins and found that these RNA-binding proteins are all localized to mitochondria where they regulate mitochondrial gene expression. Mammalian PPR proteins have diverse roles in RNA metabolism and translation that are important for mitochondrial function and cell health. To investigate the importance of RNA-binding proteins in mitochondria globally we have established new methods for massively parallel sequencing and analyses of RNase-accessible regions of human mitochondrial RNA. We have identified specific regions within mitochondrial transcripts that are bound by RNA-binding proteins. These mitochondrial protein footprints indicate that RNA-binding proteins as well as small RNAs play a significant role in the regulation of mitochondrial gene expression.

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707-C Discovery of novel small molecules that bind HCV IRES RNA by SHAPE-directed screening

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The revolution in RNA biology makes it clear that directly targeting RNA has the potential to treat many diseases. However, identification of small molecules that bind specific RNAs remains exceedingly difficult. To address this challenge, we developed a highly automated selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) assay to screen for small molecule binding to large, highly structured RNA at single nucleotide resolution. We validated this approach against the Hepatitis C Virus (HCV) internal ribosome entry site (IRES), a highly conserved RNA required for cap-independent viral translation. HCV IRES RNA is an attractive drug target that contains multiple, functionally critical, structured domains. Previous work has shown that domain IIA binds 2-aminobenzimidazole derivatives. We used SHAPE to screen a focused library of 268 2-aminobenzimidazole based compounds to detect potential binding events across all domains of HCV IRES RNA. Our analysis showed statistically significant changes in SHAPE reactivity in multiple domains of HCV IRES RNA for >15 compounds. These data suggest that automated SHAPE screening is a powerful method for identifying small molecule ligands for large, structurally complex RNAs. Furthermore, the rich array of interactions between ligands that we identified and distinct domains in HCV IRES RNA hint that RNA, in general, may comprise a new and highly targetable class of small molecule receptors.

710-C Screening of the RNase-sensitive subnuclear structures identified the Sam68 nuclear body that was built on RNA with novel protein components

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The mammalian cell nucleus contains membraneless suborganelles characterized by a distinct set of resident proteins and referred to as nuclear bodies. The nuclear bodies are thought to serve as the sites for biogenesis of various RNA species, the storage and assembly of ribonucleoprotein complexes and the retention of specific RNA species. We have been studying on the nuclear paraspeckle that is formed around the architectural NEAT1 long noncoding RNA (lncRNA) (Sasaki et al., PNAS 2009, Naganuma et al., EMBO J 2012). Here, we searched for additional nuclear bodies that were built on unidentified architectural RNAs by screening of the "RNase-sensitive nuclear bodies" using 10432 fluorescence-tagged human full-length (FLJ) cDNA clones (Goshima et al., Nat methods 2008). First, we chose 463 FLJ cDNA clones whose fluorescently tagged-protein products were localized in certain nuclear foci. Subsequently, we explored whether the respective nuclear focus was abolished or diffused upon RNase treatment after cell permeabilization. "The RNase-sensitivity screening" identified 25 tagged proteins that required RNA for their localization in distinct nuclear foci. Immunostaining of the corresponding endogenous proteins confirmed that the Sam68 nuclear body (SNB) was an RNase-sensitive structure. The above screening simultaneously identified five novel SNB proteins (SNB1-5) whose localization in SNB were also abolished by RNase treatment. RNAi of each SNB component revealed that two RNA-binding proteins (Sam68 and SNB3) were required for SNB formation. Our data argue that the SNB structural core is built on certain nuclear RNA(s) that collaborate with at least two RNA-binding proteins.

713-C Imaging trinucleotide repeat RNA in live cells using Spinach2, an RNA tag with enhanced brightness and thermostability

Rita Strack[1], *Samie Jaffrey*[1]

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Decades of research have revealed the vast array and crucial roles of RNA at all levels of gene expression. Imaging RNAs in real time in living cells is vitally important for studying these processes, but is technically challenging. A genetically encoded RNA mimic of GFP called Spinach was recently described that provides a markedly simplified approach for imaging RNAs in living systems. Spinach is an aptamer that binds a small molecule chromophore. Separately, both Spinach and the chromophore are nonfluorescent; however, when bound the Spinach-chromophore complex is brightly fluorescent, allowing specific imaging of tagged RNAs in living cells. Although powerful, Spinach suffers from limited sensitivity in mammalian systems due to low thermal stability, poor folding, and ion sensitivity. For this reason, we subjected Spinach to targeted mutagenesis and developed Spinach2. Spinach2 has enhanced brightness, thermal stability, and folding relative to Spinach, and performs robustly in both bacterial mammalian cells. We have used Spinach2 to study toxic CGG-repeat containing RNAs in a model of Fragile-X tremor ataxia syndrome and show that Spinach2 is a versatile new tool for *in vivo* imaging.

716-C Identification of novel post-transcriptional regulatory sequences

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[1]Department of Molecular Biology & Genetics, Cornell UniversityRNA 2013 Late Additions

Post-transcriptional events are essential for an organism's proper function and development, and misregulation of these events often results in cancers and other diseases in humans. Identifying sites within mRNAs that determine their post-transcriptional fates is therefore an essential step towards understanding post-transcriptional regulatory pathways. Such sites are typically found in the gene's untranslated regions (5'UTR and 3'UTRs), where they recruit trans-acting factors to control mRNA localization, decay rates, and translation efficiency. Many UTR regulatory sequences, as well as their mechanisms of action, still remain to be discovered.

One method we utilized was to use comparative genomics to identify preferentially conserved sequences in UTRs. We focused on those sequences that do not match binding sites for known trans-factors, and used traditional reporter assays in cell culture to validate multiple novel motifs. We are currently investigating the mechanisms by which a set of these sequences mediate post-transcriptional regulation. Although our approach has been successful in identifying novel regulatory sequences, it is not practical for large-scale validation of the thousands of conserved sequences observed in human UTRs.

To begin globally assessing 3'UTR sequences for regulatory ability, we developed an assay to test thousands of possible regulatory sequences in parallel. We generated large GFP reporter libraries in which random short sequences were inserted within the 3'UTR. When these reporters were integrated into the genome of cultured human cells, the GFP intensity corresponded to the post-transcriptional regulatory potential of the inserted sequence: cells with low GFP intensity contained a sequence that downregulated expression, and vice versa. We isolated cells undergoing differential expression and identified the proportions of each inserted short sequence in the different cell populations. We performed this screen in the context of three different human 3'UTRs and identified sequences that alter post-transcriptional gene regulation. We will compare this functional data to sequences conserved in human 3'UTRs. Additionally, our approach can identify sites undergoing rapid evolution without prior knowledge of the *trans*-factors. Because of the widespread impact of post-transcriptional regulation, knowing which sequences act in post-transcriptional regulation will help us to better understand human health and disease.