RNA 2013 Poster Session A Table of Contents

Use the bookmarks and navigate to the desired **Session Name**. Select the abstract and click an abstract number to open the abstract. After viewing, use the bookmarks to return to another section or scroll to see more abstracts in the session.

Non-coding and Regulatory RNAs

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 156 A – 204 A
Location: Main Hallway & Sanada Foyer

156-A Natural antisense transcripts in Neurospora crassa

Yamini Arthanari[1], *Sam Griffiths-Jones*[1], *Christian Heintzen*[1], *Susan Crosthwaite*[1]

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Antisense (AS) transcripts have been shown to play an important role in regulating the expression of genes in many organisms. Regulation of transcription, transcript splicing, stability and translation by AS transcripts has been reported. Here we report the prevalence of *cis*-AS transcripts in the model organism, *Neurospora crassa.*

RNA sequencing was performed using ABi SOLiD technology on RNA extracted from cultures grown in constant darkness at 25°C or exposed to a light or temperature pulse. The RNASeq reads were mapped to the genome and assembled into transfrags. We detected 6771 (~70%) transfrags arising from previously annotated genes that are expressed above a threshold level. Those transfrags that were not located close to a previously annotated transcript were considered to be novel transcripts. This led to the detection of over 1000 novel transcripts, only 60 of which were predicted to have significant coding potential. Further analysis led to the identification of more than 400 annotated sense/AS pairs and more than 450 novel transcripts that are expressed antisense to annotated genes. 57 and 34 novel transcripts were found to be differentially expressed in response to light and temperature respectively, approximately half of these were novel AS transcripts. The expression of several sense and AS transcripts was confirmed by RT-PCR. Hence, this study reveals the prevalence of novel *N. crassa* noncoding and AS transcripts and their expression in different environments.

159-A TDP-43 regulates cancer-associated microRNAs

Xiaowei Chen[1], Zhen Fan[5], Mengmeng Chen[6], Ruirui Kong[5], Pushuai Wen[5], Tengfei Xiao[5], Warren McGee[2], Xiaomin Chen[4], Jianghong Liu[4], Li Zhu[4], Runsheng Chen[4], Jane Wu[3]

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MicroRNAs (miRNAs) play important roles in a wide range of biological processes. Aberrant regulation of miRNA genes contributes to human diseases, including cancers. The TAR DNA binding protein 43 (TDP-43), a DNA/RNA binding protein associated with neurodegeneration, is involved in miRNA biogenesis. Here, we systematically examined miRNAs whose expression levels are regulated by TDP-43 using RNA-Seq coupled with siRNA-mediated knockdown approach. Candidate TDP-43 targeted miRNAs were validated by quantitative RT-PCR. Some of TDP-43 regulated miRNAs appear to interact with TDP-43. Alterations in isomiR patterns and miRNA arm selections following TDP-43 down-regulation indicated TDP-43 is involved in miRNA editing. We examined correlation of selected TDP-43 regulated miRNAs and their candidate target genes and their expression patterns in human cancers. Our data show that TDP-43 promotes migration of lung cancer cell by regulating expression of TDP-43 target miRNAs. We have begun to analyze the correlation of expression of TDP-43 regulated miRNAs with disease outcome. Our experiments have also begun to reveal previous unknown miRNA-target gene pairs that may play important roles in cancer and other diseases.

162-A The role of non coding RNA at sites of DNA damage in the control of genome integrity

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The DNA damage response (DDR) is a signaling pathway that arrests the proliferation of cells undergoing genotoxic events to preserve genome integrity. DICER and DROSHA are crucial ribonucleases involved in RNA interference (RNAi). Components of RNAi are thought to have evolved to preserve genome stability from the attacks of viruses and mobile genetic elements. RNAi and DDR pathways had not been demonstrated to directly interact.

We have recently shown (Francia et al. Nature 2012) in cultured human and mouse cells and in zebrafish larvae that DICER and DROSHA, but not downstream elements of the RNAi pathway, are necessary to activate the DDR upon exogenous DNA damage and oncogene-induced genotoxic stress, as studied by DDR foci formation and by DNA-damage checkpoint assays. DDR foci are sensitive to RNase A treatment, and DICER- and DROSHA-dependent RNA products are required to restore DDR foci in RNase-A-treated cells. Through RNA deep sequencing and the study of DDR activation at a single inducible DNA double-strand break (DSB), we identified site-specific DICER- and DROSHA-dependent small RNAs, named DDRNAs, with the sequence of the DNA surrounding the DSB that are required for DDR foci formation. DDRNAs, either chemically synthesized or in vitro generated by DICER cleavage, are sufficient to restore the DDR in RNase-A-treated cells, even in the absence of other cellular RNAs. Therefore, DDRNAs are a novel class of ncRNAs that play a direct role in the control of DDR activation at sites of DNA damage.

Our progresses in understanding DDRNAs biogenesis and functions will be presented.

165-A Identification and Analysis of New Genes Targeted for sRNA Regulation in Bacteria

Martha Faner[1], *Andrew Feig*[1]

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Many bacteria use trans-encoded sRNAs to respond to environmental conditions. This pathway typically uses the RNA-binding protein Hfq to facilitate the identification of target mRNAs and to promote pairing between the sRNA and its mRNA partners. This interaction subsequently leads to altered translation or targeted RNA degradation. Because *trans*-sRNAs act by imperfect base pairing they often regulate multiple mRNAs, forming a network of regulatory actions. Annotation of sRNAs in bacteria has been extensive but it has been harder to identify and validate the direct targets of sRNA regulation.

Guided by knowledge of the motifs found on mRNAs and their common location within mRNA transcripts. We have identified a collection of new regulatory circuits. While the methodology for discovering these regulated genes still reports some false positives – validation shows that roughly 1/3 of the predicted targets do in fact undergo direct regulation when studied with reporter gene constructs.

The discovery of new mRNA targets demonstrates an increased complexity of the sRNA regulatory network in bacteria. More importantly, the ability to predict new mRNA targets provides significant benefits for genomes beyond *E. coli* where less is known about the biology of regulatory RNAs and provides a means to rapidly elucidate these pathways.

168-A Alu RNAs as possible modulators of microRNA function

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Alu elements are the most abundant repetitive elements in the human genome and belong to the class of short interspersed elements (SINEs) which are non-autonomous retrotransposons. Approximatively one million copies of Alu elements pervaded the genome throughout evolution making up more than 10% of it. They derive from the 7SL RNA gene, which encodes the RNA moiety of the signal recognition particle (SRP).

Depending on the site of integration during retrotransposition, Alu elements can be embedded in coding genes and are therefore co-transcribed by RNA polymerase II (Pol II). Additionally, Alu elements can be transcribed by RNA polymerase III (Pol III), since they have partially retained internal promoter regions.

Under normal conditions free Alu RNAs accumulate at low levels. Their abundance increases dramatically under various stress conditions such as heat shock, viral infection, and exposure to toxic agents. Little is known about the molecular function of free Alu RNAs. Some studies pointed out that they inhibit transcription by direct interaction with Pol II. Other groups reported naked Alu RNAs to have a stimulatory role for protein translation, while Alu RNAs bound to protein components of the SRP inhibit initiation of translation.

Recent data suggest that Alu RNA might have direct toxic effects in the pathogenesis of age-related macular degeneration, a disease leading to progressive loss of central vision. Here, decreased protein levels of the RNAseIII enzyme Dicer (Dcr), which is required for microRNA (miRNA) biogenesis, have been associated with accumulation of Alu RNAs while miRNA expression remained unaffected. The authors claimed for a direct role of Dcr in the degradation of Alu RNAs. Our study aims to determine if Alu RNAs are in fact degraded or processed to small RNAs by Dcr and if a crosstalk between Alu RNA expression and the miRNA regulatory pathway exists.

171-A A tandem-stem RNA motif mediates X-chromosome dosage compensation in Drosophila

Ibrahim Avsar Ilik[1], Jeffrey J. Quinn[3], Filipe Tavares-Cadete[2], Plamen Georgiev[1], Yue Wan[3], Robert C. Spitale[3], Nicholas Luscombe[2], Howard Y. Chang[3], Asifa Akhtar[1]

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Long non-coding RNAs (lncRNAs) are emerging as important regulators of chromatin state and transcription in eukaryotic cells. They contribute to the regulation of single genes or whole chromosomes and can influence the 3-D structure of large genomic regions. Due to their length, which typically is in the range of kilobases, it is difficult to determine functional domains in these lncRNAs and therefore understand their exact contributions to transcriptional regulation. A prime example of lncRNAs regulating chromosome-wide gene expression is X-chromosomal dosage compensation. Sexually dimorphic organisms often correct for the unequal distribution of sex chromosomes in males and females in order to have a balanced gene expression between the sex chromosomes and the autosomes. This process, called X-chromosomal dosage compensation, requires the action of lncRNAs all the way from Humans to Drosophila. Interestingly though, the underlying mechanisms lie at the two opposing ends of the transcriptional spectrum: In humans Xist lncRNA (~17kb) plays a central role in repressing one of the two X-chromosomes in females; whereas in Drosophila, two redundant lncRNAs, roX1 (~3.7kb) and roX2 (~0.6kb) are necessary for the transcriptional up-regulation of the single X-chromosome in male flies. Dosage compensation in Drosophila is mediated by the Male-Specific Lethal (MSL) complex, which together with roX lncRNAs coats the male X chromosome and acetylates histone H4 lysine 16. Genetic evidence suggests that roX RNAs are particularly important for targeting the MSL complex to the X-chromosome, however, biochemical data detailing roX RNA – MSL complex interactions and how targeting of the MSL complex specifically to the X-chromosome can be aided by roX RNAs has been lacking.

Here, by using UV cross-linking followed by deep sequencing we show that two enzymes in the MSL complex, MLE RNA helicase and MSL2 ubiquitin ligase, bind evolutionarily conserved domains containing tandem stem-loops in roX1 and roX2 RNAs *in vivo*. These domains constitute the minimal RNA unit present in multiple copies in diverse arrangements for nucleation of the MSL complex. MLE binding to these domains is bimodal with distinct ATP-independent and ATP-dependent behavior. Importantly, we show that cooperative interaction of the different roX RNA domains is essential because combinatorial but not single mutations result in loss of dosage compensation and consequently male-specific lethality. Our study reveals that repetitive structural motifs in lncRNAs provide plasticity during multi-protein complex assembly to ensure efficient spreading *in cis* or *trans* along chromosomes.

174-A Circular RNAs are Abundant, Conserved and Linked to ALU Repeats

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Formerly thought to be errors of splicing, endogenous circular RNAs have recently been shown by our group and other to be abundant and conserved. While circular RNAs have been identified by bioinformatic analysis, we sought to directly identify circular RNAs through an unbiased biochemical approach. Toward that end, we performed high-throughput sequencing (RNA-seq) on libraries derived from human and murine cells prepared from ribosome-depleted total RNA with or without digestion with RNA exonuclease. We identified >25,000 circular RNA species in human fibroblasts. Circular RNAs were usually composed entirely of exonic sequence. These exonic, circular RNAs (ecricRNAs) were identified by the presence of non-colinear exons (a "backsplice") and enrichment by exonuclease treatment. These species were validated as circular through biochemical approaches, and were considerably more stable than associated linear mRNAs. Expression of ecircRNAs ranged from very rare to highly abundant, with circular molecules exceeding the number of linear mRNA molecules by >10-fold for some transcripts. We did not identify ecircRNAs associated with ribosomes. Application of this method to murine testis RNA identified 69 ecircRNAs in precisely orthologous locations to human circular RNAs. We identified paralogous kinases, *HIPK2* and *HIPK3,* that produce abundant ecircRNA from their second, AUG-containing exon in both humans and mice. We noted degradation of circular RNAs by siRNAs, suggesting these species regulate gene expression by acting as competing endogenous RNAs. Analysis of backsplice sites utilized by ecircRNAs revealed shared features of circularized exons, including long bordering introns often containing complementary ALU repeats. EcircRNAs identified by this method were expressed in human cancer, with differential expression among tumor types. These data show that ecircRNAs are abundant, stable, and conserved products of RNA splicing that appear to regulate gene expression.

177-A The double stranded RNA transcriptome of E. coli reveals novel antisense RNAs

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Antisense RNAs are transcribed from the DNA strand opposite another gene and have perfect complementarity to the corresponding sense mRNA. Advances in high-throughput transcriptome analyses have revealed hundreds of antisense RNAs for many bacteria, although few have been characterized, and the number of functional antisense RNA remains unknown. We developed a method to identify potentially functional antisense RNAs in *Escherichia coli*. Most mechanisms of regulation via antisense RNAs require a RNA/RNA interaction with its target RNA. We hypothesized that a functional antisense RNA would be found in a double stranded RNA (dsRNA), duplexed with its cognate mRNA in a single cell. Therefore, we developed a method of enriching dsRNAs from total RNA by immunoprecipitating with a monoclonal antibody specific for dsRNA. Total RNA and dsRNA fractions from RNase III wild-type and mutant strains were converted to cDNA libraries, deep sequenced and many novel, potentially functional antisense RNAs were identified. The sense strand of many newly identified dsRNAs are known, functional sRNAs including: DsrA, Spot42, MicA, MicM, CyaR, ArcZ, MgrR and RydC. In addition, we identified many asRNAs encoded opposite of 5' ends of mRNAs and gene junctions of operons. Northern blot analyses have confirmed the presence of over 20 of the novel antisense RNAs and functional and mechanistic characterization is underway.

180-A MiR-19 and miR-155 role in oncogene-induced senescence bypass

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Cancer establishment and progression is a multi-step process that requires various concerted modifications. Fortunately, cells have evolved many different mechanisms to counteract tumorigenesis. Oncogene-induced senescence (OIS) is one of these mechanisms and it has been shown to be triggered early on in tumorigenesis. Its establishment is mainly carried through the activation of two important tumor suppressor pathways: the p53/p21 pathway and the p16/RB pathway. Thus in cancer progression, key players in these pathways must be altered in order to bypass this barrier.

Leukemia is often characterized by an over-expression of STAT5A. The latter is known to induce sets of genes that are antagonistic leading to either cell proliferation or senescence. Accumulating reports also provide evidence that microRNAs (miRNAs) are upregulated in various leukemias suggesting an oncogenic role for these. We propose that normal STAT5 signaling includes the regulation of the tumor suppressor SOCS1 that prevents uncontrolled cell proliferation and triggers senescence in part by regulating p53. We think that during leukemia, constitutively active STAT5A induces the oncomirs (oncogenic miRNAs) miR-19 and miR-155 resulting in down-regulation of SOCS1 by the miRNAs and subsequent bypass of the oncogene-induced senescence.

In order to study the role of the oncomirs in leukemia we used microRNA sponges that inhibit miRNA activity by titrating their levels. Our results show that sponges against miR-19 or miR-155 stabilized SOCS1 and enhanced p53 activation as shown by measuring p53 phosphorylation at serine 15 and its transcriptional target p21. Although the development of therapeutics to induce miRNA down-regulation is currently underway, or work suggests that inhibition if miR-19 and miR-155 can reactivate endogenous tumor suppressors in leukemia.

183-A Structure and function of Zucchini endoribonuclease in piRNA biogenesis

Osamu Nureki[1], *Hiroshi Nishimasu*[1], *Hirotsugu Ishizu*[1], *Mikiko Siomi*[1]

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PIWI-interacting RNAs (piRNAs) silence transposons to maintain the integrity of the genome in animal germlines. piRNAs are divided into primary and secondary piRNAs depending on the biogenesis machinery. Primary piRNAs are processed from long non-coding RNA precursors transcribed from piRNA clusters in the genome through the primary processing pathway. Although the existence of a ribonuclease participating in this pathway has been anticipated, its molecular identity remains elusive. Here, we show that Zucchini (Zuc), a mitochondrial phospholipase D (PLD) superfamily member, is an endoribonuclease essential for primary piRNA biogenesis. We solved the crystal structure of *Drosophila melanogaster* Zuc (DmZuc) at 1.75-Å resolution. The structure revealed that DmZuc has a positively charged, narrow catalytic groove at the dimer interface, which could accommodate a single-stranded, but not a double-stranded, RNA. DmZuc and the mouse homolog MmZuc (also known as Pld6 and MitoPLD) exhibited endoribonuclease activity for single-stranded RNAs *in vitro*. The RNA cleavage products bear a 5'-monophosphate group, a hallmark of mature piRNAs. Mutational analyses showed that conserved active-site residues of DmZuc are critical for ribonuclease activity *in vitro*, and for piRNA maturation and transposon silencing *in vivo*. We propose a model for piRNA biogenesis in animal germlines, in which Zuc endoribonuclease plays a key role in primary piRNA maturation.

186-A Degradation of ribosomal RNA and ribosomal proteins constitute separate pathways of ribophagy

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Nutritional deprivation leads to severe changes in cell homeostasis, ultimately promoting destruction of cellular components and organelles to prevent death. Recently, a deubiquitination-dependent selective degradation of ribosomal proteins in yeast vacuoles, which is activated by nitrogen starvation and termed ribophagy, has been reported. We describe a separate ribophagy mechanism, wherein robust fragmentation of mature ribosomal RNA in autophagic yeast cells is independent of protein degradation. This ribosome recycling mechanism is highly specific, requires a subset of autophagic regulators, Atg proteins, is dependent on the TOR (target of rapamycin) regulatory pathway, but proceeds efficiently in the absence of the Ubp3-Bre5 complex required for the ribophagic degradation of ribosomal proteins. This indicates that the concurrent response on the protein part of ribosomal subunits is not a prerequisite. RNA cleavages take place on intact ribosomes in the cytoplasm and are carried out predominantly, but not solely, by the vacuolar ribonuclease Rny1, which is released from the vacuole to the cytosol upon activation of autophagy. Endonucleolytic cleavages generate specific and stable RNA fragments, which are further digested by exonucleases, including Nuc1, 5'-3' exonuclease Xrn1 and the 3'-5' exosome complex. In contrast, components of both 18S and 25S "non-functional rRNA decay" (NRD), that eliminates malfunctionning ribosomes, are not required for rRNA destruction during autophagy. The existence of the autonomous rRNA ribophagic pathway substantiates the importance of ribosome breakdown to survive the shortage of nutrients.

189-A RNA Structure and Ligand Interactions Probed by Strategically Positioned 15N-Labels

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To explore folding and ligand recognition of metabolite-responsive RNAs is of major importance to comprehend gene regulation by mRNA riboswitches. On the secondary structure level, folding events often lead to changes in the H-bond mediated Watson-Crick base pairing pattern. The individual base pairs can be monitored by the NMR spectroscopic HNN-COSY experiment which detects and quantifies magnetically active ^{15}N nuclei of the H-bond donor and acceptor.

Here, we present the synthesis of all four ribonucleoside phosphoramidites carrying single ^{15}N-labels at the pyrimidine-3 or purine-1 positions. Their site-specific introduction into RNA has been achieved by solid-phase synthesis using a combination of 2'-*O*-TOM and 2'-*O*-TBDMS RNA chemistry.

To furthermore demonstrate the power of the labels for NMR spectroscopic applications, we investigate pseudoknot folding and ligand interaction of transcriptionally [1] as well as translationally acting $preQ_1$ class-I aptamers in a Mg^{2+} and temperature dependent manner. The study provides detailed insights into the ligand recognition mode of $preQ_1$ riboswitches.

[1] T. Santner, U. Rieder, C. Kreutz, R. Micura; Pseudoknot preorganisation of the $preQ_1$ class I riboswitch; J. Am. Chem. Soc. 2012, 11928-11931.

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192-A Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells

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Many long non-coding RNA (lncRNA) species have been identified in mammalian cells, but the genomic origin and regulation of these molecules in individual cell types is poorly understood. We have generated catalogs of lncRNA species expressed in human and murine embryonic stem cells (ESCs) and mapped their genomic origin. A surprisingly large fraction of these transcripts (>85%) originate from divergent transcription at promoters or enhancers of active protein-coding genes. The divergently transcribed lncRNA/mRNA gene pairs exhibit coordinated changes in transcription when ESCs are differentiated into endoderm. Down-regulation of a divergently transcribed lncRNA by RNAi reduces the efficiency of differentiation of hESCs into the endoderm. Our results reveal that transcription of most lncRNA genes is coordinated with transcription of protein-coding genes.

195-A RNA polymerase-binding elements attenuating transcription in E. coli

Nadia Tukhtubaeva[1], Vitaliy Epshtein[4], Bob Zimmermann[3], Meghan Lybecker[3], Ivana Bilusic[2], Evgeny Nudler[4], Renee Schroeder[2]

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Transcription is a highly controlled step of gene expression. A number of diverse RNA-based transcription regulatory mechanisms have been discovered to date. However, in contrast to numerous well-studied protein factors, there are only few known regulatory RNAs tuning transcription by a direct interaction with the RNA polymerase machinery. Addressing the question if such type of transcriptional riboregulation could be a frequent phenomenon in bacteria, we combined *E. coli* RNAP - genomic SELEX with next-generation sequencing. The obtained data reveal a large group of polymerase binding RNA elements (PBEs) located within diverse coding sequences that bind RNAP with high affinity. Using different *in vitro* and *in vivo* techniques we demonstrate that a subgroup of these PBEs attenuate transcription of the nascent RNA by promoting premature termination events under relevant biological conditions. Our findings let us propose a novel and potentially widespread mechanism of transcriptional regulation by the transcribing RNA itself. Detailed single-molecule mechanistic elucidation of the process is underway.

198-A Rat mir-155 generated from the lncRNA Bic is 'hidden' in the alternate genomic assembly and reveals the existence of novel mammalian miRNAs and clusters

Paolo Uva[1], Letizia Da Sacco[3], Manuela Del Cornò[2], Paola Sestili[2], Sandra Gessani[2], Antonella Baldassarre[4], Massimiliano Orsini[1], Andrea Masotti[4], Alessia Palma[5], Mattia Locatelli[1]

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MicroRNAs (miRNAs) are a class of small noncoding RNAs acting as post-transcriptional gene expression regulators in many physiological and pathological conditions. During the last few years, many novel mammalian miRNAs have been predicted experimentally with bioinformatics approaches and validated by next-generation sequencing. Although these strategies have prompted the discovery of several miRNAs, the total number of these genes still seems larger. Here, by exploiting the species conservation of human, mouse, and rat hairpin miRNAs, we discovered a novel rat microRNA, mir-155. We found that mature miR-155 is overexpressed in rat spleen myeloid cells treated with LPS, similarly to humans and mice. Rat mir-155 is annotated only on the alternate genome, suggesting the presence of other "hidden" miRNAs on this assembly. Therefore, we comprehensively extended the homology search also to mice and humans, finally validating 34 novel mammalian miRNAs (two in humans, five in mice, and up to 27 in rats). Surprisingly, 15 of these novel miRNAs (one for mice and 14 for rats) were found only on the alternate and not on the reference genomic assembly. To date, our findings indicate that the choice of genomic assembly, when mapping small RNA reads, is an important option that should be carefully considered, at least for these animal models. Finally, the discovery of these novel mammalian miRNA genes may contribute to a better understanding of already acquired experimental data, thereby paving the way to still unexplored investigations and to unraveling the function of miRNAs in disease models.

201-A Regulation of transcription by Pol II-binding RNA aptamers

Adam Weiss[1], Maximilian Radtke[1], Frederike von Pelchrzim[1], Jennifer Boots[1], Bob Zimmermann[1], Marek Zywicki[2], Katarzyna Matylla-Kulinska[1], Renée Schroeder[1]

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Transcription by RNA polymerase II (Pol II) is the first step in eukaryotic gene expression and one of the most important ones to regulate. As abundance of non-coding RNAs with diverse functions have been identified, it has become clear that RNAs regulate every step in gene expression including transcription. To investigate the regulatory potential of the human transcriptome, we performed Genomic SELEX with Pol II as a bait and identified many high-affinity RNA aptamers (Polymerase-binding elements or PBEs). PBEs can be pulled down with Pol II from HeLa cells and they abolish transcription when fused to a reporter gene in an *in vivo* expression system. Interestingly, many PBEs are located in repetitive regions of the human genome, with most prominent ACRO1 satellites, previously uncharacterized repeat elements that cluster in the pericentromeric region of chromosome 4 and can be found on chromosomes 1, 2, 19 and 21 as well. Another class of PBE-containing repeats are the L1 retrotransposons, which had been shown to inhibit their own expression by an unknown mechanism. Our results suggest a novel way of *in-cis* transcription regulation by RNAs, wherein nascent transcripts bind Pol II to interfere with elongation.

204-A Scaffold function of long noncoding RNA HOTAIR in protein ubiquitination

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Although mammalian long noncoding (lnc)RNAs are best known for modulating transcription, their post-transcriptional influence on mRNA splicing, stability, and translation is emerging. Here we report a post-translational function for the lncRNA *HOTAIR* as an inducer of ubiquitin-mediated proteolysis. *HOTAIR* associated with E3 ubiquitin ligases bearing RNA-binding domains, Dzip3 and Mex3b, as well as with their respective ubiquitination substrates, Ataxin-1 and Snurportin-1. In this manner, *HOTAIR* facilitated the ubiquitination of Ataxin-1 by Dzip3 and Snurportin-1 by Mex3b in cells and *in vitro*, and accelerated their degradation. *HOTAIR* levels were highly upregulated in senescent cells, causing rapid decay of targets Ataxin-1 and Snurportin-1, and preventing premature senescence. These results uncover a role for a lncRNA, *HOTAIR*, as a platform for protein ubiquitination.

Mechanisms of RNA interference

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 207 A – 222 A
Location: Main Hallway & Sanada Foyer

207 A **Argonaute-associated factors required for translational repression in plant RNA silencing**
210 A **Human Dicer caught in the act via single-molecule fluorescence spectroscopy**
213 A **Real-time observation of target binding and dissociation of Argonaute-guide complex**
216 A **crRNA-guided R-loop formation and the architecture of the Type I-C Cascade**
219 A **Functional characterization of DCL1 and DCL2 proteins from Medicago truncatula**
222 A **The CCR4-NOT complex releases PABP from silenced miRNA targets in the absence of deadenylation**

207-A Argonaute-associated factors required for translational repression in plant RNA silencing

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In *Arabidopsis thaliana*, microRNAs (miRNAs) are loaded in ARGONAUTE 1 (AGO1) as part of RNA induced silencing complexes (RISCs) to regulate target messenger RNAs (mRNAs) via base-pairing. AGO1 possesses an intrinsic endonuclease activity responsible for the 'slicing' of mRNA targets, an activity that is abrogated when bulges or mismatches face nucleotides 10-11 of the miRNAs. Such mismatches promote in turn alternative forms of target repression such as the translational inhibition and/or decay of the mRNAs. Intriguingly, the fact that in *Arabidopsis* most of the miRNAs regulates their target mRNAs via perfect or near-perfect complementarity has contributed to the widespread belief that plant miRNAs, unlike their animal counterparts, exert their effect mostly through target mRNA slicing. Yet, a previous work notably carried out in our lab suggests that these miRNAs can concurrently slice and transnationally inhibit a given pool of mRNAs. This raises the fundamental question of how slicing is avoided during translational inhibition. One possibility is that translational repressor proteins actually associate to AGO1 in order to change further the fate of the target mRNAs. Following this hypothesis, we have biochemically isolated three AGO1-associated factors. Here, we show that these proteins genetically interact with AGO1; they do not affect the miRNAs biogenesis, nor the stability of the main silencing factors, but clearly change the mode of action of AGO1. Indeed, upon deletion of these proteins, the slicing activity of AGO1 is greatly improved, favouring the cleavage of the target mRNAs. Surprisingly, this property also gives to these mutant plants a stronger resistance upon infection by the *Tobacco rattle virus* (TRV). Together, our results revealed the existence of three AGO1 partners that seem important to modulate the slicing activity of AGO1. As multiple pools of AGO1 co-exist in *Arabidopsis*, we propose that these factors act as guides and drive AGO1 toward a specific protein complex in which the translational inhibition is favoured over slicing.

210-A Human Dicer caught in the act via single-molecule fluorescence spectroscopy

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The microRNAs are endogenous small non-coding RNAs generated through a series of enzymatic processing steps. After an RNA transcript is processed by the Drosha complex, the precursor miRNA (pre-miRNA) is exported to the cytoplasm where it is cleaved by the Dicer complex. The latter contains Dicer and several non-catalytic accessory proteins such as TRBP and PACT. Despite the discovery of the key actors in the miRNA biogenesis, the molecular basis of miRNA maturation remains unclear. In order to unveil the molecular mechanisms of pre-miRNA processing by the Dicer complex, we have developed a single-molecule technique by combining fluorescence spectroscopy with a protein complex pull-down assay. Using this new technique, we have shown step by step the action mechanism of human Dicer and its cofactors – from RNA binding and cleavage to product release. Our single-molecule data highlights novel coordination between Dicer and its cofactors.

213-A Real-time observation of target binding and dissociation of Argonaute-guide complex

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Argonaute (Ago) is the key component of the RISC (RNA-induced silencing complex) which is an effector of small RNA(20–30nt)-based gene regulation. In the regulatory process, target recognition by Ago-guide is important for controlling gene expression vigorously. Target dissociation is also crucial for the recycling of Ago in the regulation. Here, by using single-molecule fluorescence resonance transfer (FRET) assays and *Thermus thermophilus* Ago (*Tt*Ago), we monitor the target binding/dissociation kinetics of Ago-guide and reveal that different regions of guide-target base pairing have different roles in their binding and dissociation.

216-A crRNA-guided R-loop formation and the architecture of the Type I-C Cascade

Ki Hyun Nam[1], *Ailong Ke*[1]

[1]**Cornell University**

CRISPR-Cas system serves as an RNA-based immune system against invasive genetic elements. In several Type I CRISPR-Cas systems, the subtype-specific Cas proteins assemble around the CRISPR RNA (crRNA) to form Cascade, which recognizes the target ds-DNA in a sequence specific fashion. Here we reveal the discrete biochemical steps leading to the target DNA recognition by the *B. halodurans* Type I-C Cascade, report its low resolution EM structure, and the crystal structure analysis of the scaffolding protein Csd2. We provide evidence that I-C Cascade actively promotes the base-pairing between crRNA spacer and the target ds-DNA, forming the R-loop structure. The architecture of *B. halodurans* Type I-C Cascade resembles *E. coli* Type I-E Cascade, with minor architectural differences. The Csd2 crystal structure containss a ferredoxin fold and a positively charged groove, which upon oligomerization forms an extended nonspecific binding surface for crRNA or crRNA/DNA duplex. Mutagenesis guided by the crystal structure and EM docking further provides insights about the nucleic acid binding and oligomerization mechanisms in Csd2. Overall, our results define the molecular function of Type I-C Cascade.

219-A Functional characterization of DCL1 and DCL2 proteins from Medicago truncatula

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In eukaryotic organisms several types of 21-24 nucleotides long RNA molecules (called small regulatory RNA; srRNA) are involved in the sequence-specific regulation of numerous biological processes, including developmental and stress response pathways or pathogen defense. This widespread mechanism of RNA-mediated regulation is facilitated by a range of proteins which participate in processing of srRNA precursors, and enable functioning of the mature products. Among them ribonuclease III Dicer was identified as the key enzyme involved in the last step of srRNA biogenesis. Mammals and many other animals use a single Dicer enzyme to generate a complex spectrum of srRNAs. In contrast, there are several Dicer-like proteins (DCL) typically found in plants. In *Arabidopsis thaliana* four DCL proteins were identified (AtDCL1-4). The consecutive studies demonstrated that each AtDCL protein is predominantly specialized in the production of different class of small RNAs. AtDCL1 mainly produces 21 nt long microRNAs, while the products of AtDCL2, AtDCL3, and AtDCL4 are various small interfering RNAs (siRNAs) of 22, 24, and 21 nucleotides in length, respectively. However, some functional overlaps for Arabidopsis DCL proteins were also showed. Considerably less information concerning Dicer-like proteins from other plants is available.

In order to increase our knowledge on DCL proteins in legume plants we attempted to determine how many members of a DCL family are encoded in the genome of *Medicago truncatula*. We obtained the full-length cDNAs coding for MtDCL1 and MtDCL2 proteins and determined the profile of their genes expression in different plant tissues and at different developmental stages. Next, we produced the peptides representing the "catalytic cores" of both proteins (MtDCL1-CC and MtDCL2-CC) and characterized *in vitro* their enzymatic activity and substrate specificity. Finally, we compared the enzymatic activity of MtDCL1-CC and MtDCL2-CC with the activity of two commercially available enzymes: human Dicer and parasitic protozoan *Giardia intestinalis* Dicer. The former is composed of a full set of functional domains typically found in Dicer-type proteins, while the latter can be classified as a minimal Dicer, similar to MtDCL1- and MtDCL2-CC.

222-A The CCR4-NOT complex releases PABP from silenced miRNA targets in the absence of deadenylation

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GW182 family proteins interact with Argonaute proteins and are required for the translational repression, deadenylation and decay of miRNA targets. To elicit these effects, GW182 proteins interact with PABP and the CCR4-NOT deadenylase complex. Although the mechanism of miRNA target deadenylation is relatively well understood, how GW182 proteins repress translation is not known. Here, we demonstrate that GW182 proteins decrease the association of PABP with silenced targets in the absence of deadenylation. We further show that the dissociation of PABP contributes approximately 2-fold to repression, indicating that additional repressive mechanisms are used by miRISCs to achieve maximal target silencing. Remarkably, PABP dissociation requires the interaction of GW182 proteins with the CCR4-NOT complex but not with PABP. Accordingly, NOT1, POP2 and CCR4 cause dissociation of PABP from bound mRNAs in the absence of deadenylation. Our findings indicate that the recruitment of the CCR4-NOT complex to miRNA targets by GW182 proteins releases PABP from the mRNA poly(A) tail, thereby facilitating translational repression and deadenylation. To gain further insight into the mechanism of PABP release by the CCR4-NOT complex, we are currently investigating which subunit(s) of the CCR4-NOT complex contacts directly PABP and is responsible for PABP release. Given the central role of the CCR4-NOT complex in post-transcriptional mRNA regulation, we anticipate that this novel activity of the complex will contribute to the translational repression of a large variety of different mRNAs.

RNA and Epigenetics

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 225 A – 231 A
Location: Main Hallway & Sanada Foyer

225 A **The Igf2as transcript is exported into the cytoplasm and is associated with polysomes**

228 A **Transcriptome-wide Analysis of the m6A Landscape Reveals Pervasive Adenosine Methylation in 3' UTRs and near Stop Codons**

231 A **Mmi1, an RNA-binding protein, mediates heterochromatin gene silencing by recruiting the nuclear exosome to long non-coding RNAs in fission yeast**

225-A The Igf2as transcript is exported into the cytoplasm and is associated with polysomes

Carolina Duart-Garcia[1], *Martin Braunschweig*[1, Tosso Leeb1]

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Murine insulin-like growth factor 2 antisense (*Igf2as*) transcripts originate from the opposite strand of the same *Igf2* locus as the *Igf2* sense mRNA. *Igf2as* transcripts are located in a cluster of imprinted genes together with *Igf2*, Insulin 2 (*Ins2*) and *H19* genes on chromosome 7. Loss of imprinting of *IGF2* locus in humans is associated with Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) as well as with Wilm's tumor and colorectal cancer. We developed a RNA-FISH protocol to detect and determine the location of *Igf2as* and *Igf2* transcripts. The results from the RNA-FISH were confirmed with quantitative real-time PCR and clearly indicate that *Igf2as* transcripts are predominantly located in the cytoplasm of C2C12 cells. A polysome association study was used to determine if the *Igf2as* had coding potential. We showed that *Igf2as* sedimented with polysomes in a sucrose gradient. The cellular localization of *Igf2as* transcripts together with polysome fractionation analysis provides compelling evidence that *Igf2as* is protein coding. We performed expression analysis of *Igf2as* transcripts in muscle, brain and liver tissues at different stages of development. The results showed high *Igf2as* expression in tissues of fetuses and newborns. Contrary, in adult tissues the *Igf2as* expression was low. These expression patterns indicate that *Igf2as* plays a role in the early developmental stages. To study the function of the new putative protein we are using two approaches. First, we are overexpressing *Igf2as* in transfected C2C12 cells to investigate its effects and to characterize putative proteins. Second, we perform an RNA-Seq experiment with RNA from primary muscle cells from DMR1-U2 knockout mice compared to wild type cells. Differentially expressed transcripts will be used to conduct a pathway analysis indicating potential networks in which *Igf2as* transcripts may be involved. Results from these experiments will be presented and discussed.

228-A Transcriptome-wide Analysis of the m6A Landscape Reveals Pervasive Adenosine Methylation in 3' UTRs and near Stop Codons

Kate Meyer[1], Yogesh Saletore[1], Paul Zumbo[1], Olivier Elemento[1], Christopher Mason[1], Samie Jaffrey[1]

[1]Weill Medical College, Cornell University

Methylation of the N^6 position of adenosine (m^6A) is a post-transcriptional RNA modification whose prevalence and physiological relevance are poorly understood. The recent discovery that *FTO*, an obesity risk gene, encodes an m^6A demethylase implicates m^6A as an important regulator of physiological processes. Here we show that m^6A is a highly prevalent base modification which exhibits tissue-specific regulation and is markedly increased throughout brain development. Using a novel method for transcriptome-wide m^6A localization termed MeRIP-Seq, we identify mRNAs of 7,676 genes which contain m^6A, indicating that m^6A is a common modification of mRNA. We find that m^6A sites are enriched near stop codons and in 3' UTRs, and we uncover an association between the presence of m^6A residues and microRNA binding sites within 3' UTRs. These findings are the first global identification of the transcripts that are substrates for adenosine methylation and reveal novel insights into the epigenetic regulation of the mammalian transcriptome.

231-A Mmi1, an RNA-binding protein, mediates heterochromatin gene silencing by recruiting the nuclear exosome to long non-coding RNAs in fission yeast

Andre Verdel[1], Edwige Hiriart[1], Michael Reuter[3], Benoit Gilquin[1], Ravi Sachidanandam[2], Ramesh Pillai[3], Leila Touat-Todeschini[1]

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RNA mediates epigenetic modifications and drives transcriptional gene silencing in a broad range of eukaryotes. In the fission yeast *Schizosaccharomyces pombe*, heterochromatic gene silencing requires a close interplay between RNAi-dependent and -independent processes. However, while the RNAi-dependent process is relatively well understood, the RNAi-independent one remains largely unknown. Our studies show that the RNA-binding protein Mmi1 and its associated RNA surveillance machinery, which are known to play a central role in fission yeast sexual differentiation control, are also involved in the deposition of the heterochromatin H3K9 methylation mark and the recruitment of RNAi machinery at specific meiotic genes. In addition and quite unexpectedly, they reveal that Mmi1 also acts at non-coding regions to mediate heterochromatic gene silencing in parallel of RNAi. We will present our latest data defining this new RNA-based RNAi-independent epigenetic silencing mechanism.

Small RNAs

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 234 A – 255 A
Location: Main Hallway & Sanada Foyer

234 A **RNA-binding proteins vs. microRNAs: how IGF2BP1 modulates tumor-suppressive microRNA-action in tumor cells**
237 A **Piwi-Interacting RNAs Protect DNA Against Loss During Oxytricha Genome Rearrangement**
240 A **miRNA degradation during C. elegans development**
243 A **Novel NFL mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis**
246 A **DmGTSF1 is essential for effective retrotransposon silencing by Piwi in Drosophila ovarian somatic cells**
249 A **Withdrawn**
252 A **A proteomic screen identifies novel regulators of micro-RNA biogenesis**
255 A **The novel Tetrahymena gene COI12 is crucial for siRNA loading into the Argonaute protein Twi1p**

234-A RNA-binding proteins vs. microRNAs: how IGF2BP1 modulates tumor-suppressive microRNA-action in tumor cells

Bianca Busch[1], *Juliane Braun*[1], *Stefan Hüttelmaier*[1]

[1]Institute of Molecular Medicine,Section for Molecular cell Biology, Martin-Luther-University, Halle (Saale), Germany.

The RNA binding protein IGF2BP1 (insulin-like growth factor 2 mRNA binding protein) is highly expressed during embryonic development and is often de novo synthesized in cancer. This trait is correlated with tumor progression and lower patient survival rates. In the cytoplasm, the protein supports the expression of oncogenic factors like MYC, PTEN or ZEB1 and represses the mRNA translation of MAPK4 and ACTB. Through this post-transcriptional gene regulation IGF2BP1 promotes cell proliferation, migration and invasion of tumor-derived cells. Most interestingly, 'aggressive' tumor cells frequently express IGF2BP1 mRNA with shortened 3'untranslated region (UTR). These shorter isoforms usually result from alternative cleavage and poly-adenylation. Notably, IGF2BP1 comprises four APA-signals in an approximately 6.7kb long 3'UTR. The use of APA-signals eliminates large parts of the 3'UTR and thus shortens transcripts. This consequently causes the loss of inhibitory microRNA targeting 3'-UTR. In the case of IGF2BP1, inhibitory microRNAs include the let-7 family, suggesting regulation of oncofetal IGF2BP1 expression by tumor-suppressive microRNAs.

To identify additional regulatory microRNAs targeting the IGF2BP1 3'UTR in tumor-derived cells, we used the complete 3'UTR of IGF2BP1 as a bait in an *in vitro* microRNA trapping RNA affinity purification assay, termed miTRAP. Next-generation sequencing identified co-purified microRNAs and confirmed the association of let-7 family members, but also identified various candidate microRNAs regulating IGF2BP1 expression. Significantly, these data indicate the microRNA-family 181 as putative strong posttranscriptional regulators of IGF2BP1 expression. Several of the identified microRNAs were previously described as tumor-suppressive microRNAs, e.g. miR-181-family and miR-191, -196, -203, -205, -24-3p and miR-34a. We hypothesize that IGF2BP1 downregulation is partly responsible for the tumor-suppressive nature of these microRNAs. Additionally, the transcription of some of these microRNAs is reduced by the oncogenes MYC and ZEB1. By upregulating the expression of both these oncogenes, IGF2BP1 may inhibit the expression of the same tumor suppressive microRNAs which we found to target its mRNA.

In brief, we have identified powerful regulatory feed-back loops potentially modulating tumor progression. Current studies focus on quantifying the role of IGF2BP1 3'UTR length as a possible oncogenic 'break' in these loops and understanding the role of these candidate microRNAs in suppressing the oncogenic function of IGF2BP1.

237-A Piwi-Interacting RNAs Protect DNA Against Loss During Oxytricha Genome Rearrangement

Wenwen Fang[1], Xing Wang[1], John Bracht[1], Mariusz Nowacki[2], Laura Landweber[1]

[1]Princeton University; [2]Bern University

Genome duality in ciliated protozoa offers a unique system to showcase their epigenome as a model of inheritance. In *Oxytricha,* the somatic genome is responsible for vegetative growth, while the germline contributes DNA to the next sexual generation. Somatic nuclear development eliminates all transposons and other "junk DNA", which constitute ~95% of the germline. We demonstrate that Piwi-interacting small RNAs (piRNAs) from the maternal nucleus can specify genomic regions for retention in this process. *Oxytricha* piRNAs map primarily to the somatic genome, representing the ~5% of the germline that is retained. Furthermore, injection of synthetic piRNAs corresponding to normally-deleted regions leads to their retention in subsequent generations. Our findings highlight small RNAs as powerful transgenerational carriers of epigenetic information for genome programming.

240-A miRNA degradation during C. elegans development

Gert-Jan Hendriks[1], *Dimosthenis Gaidatzis*[1], *Helge Grosshans*[1]

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miRNAs are small non-coding RNAs that regulate gene expression at the posttranscriptional level. These endogenous small RNAs have been shown to be involved in numerous physiological and pathological processes in many different organisms. Their biogenesis has been widely studied and the main factors involved are conserved among species. Mature miRNAs can act on their targets by either translational repression or inducing transcript degradation. miRNAs can be specifically degraded and typical half-lives of miRNAs vary from several hours to days. In *C. elegans*, miRNAs are important players in the heterochronic pathway that regulates larval development. A strict regulation of transcription as well as biogenesis has been shown to be responsible for this tight control of mature miRNA levels. To study the impact of regulated miRNA degradation on miRNA levels during *C. elegans* development we performed small RNA sequencing at hourly intervals spanning two larval transitions. We found that a number of miRNAs are rapidly and extensively degraded at specific timepoints with half-lives of down to approximately one hour. In a particularly striking example we observed a >40-fold down-regulation of a specific miRNA over six hours. The factors that are involved in this rapid degradation as well as the functional relevance of this tight regulation are currently being investigated.

243-A Novel NFL mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis

Muhammad Ishtiaq[1], *Danae Campos-Melo*[1], *Kathryn Volkening*[1], *Michael Strong*[1]

[1]University of Western Ontario

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease in which the low molecular weight neurofilament (NFL) mRNA level is suppressed in motor neurons. Based on previous findings, we hypothesized that microRNAs (miRNAs) will play a central role in NFL transcript stability, and may lead to the decrease in NFL mRNA. MiRNAs are small 20-25 nucleotide molecules and act as post transcriptional gene regulators by targeting 3' untranslated region (UTR) of mRNA resulting in mRNA decay or translational silencing. We profiled the expression of small RNA from spinal cord lysates from sporadic ALS and neurologically intact controls and determined which of these small RNA were alternatively expressed in ALS. Out of 304 differentially expressed small RNA determined to be miRNA, a total of 42 (30 known, 12 novel) were predicted to target NFL mRNA (determined by targetscan and manual screens). Of these, 12 were found to be down-regulated in sporadic ALS and the remaining were up-regulated compared to neurologically intact controls. Real time PCR analyses confirmed that the majority of these miRNAs were up-regulated in ALS except block294889, block1336549, block11865845, block4652280, block 2403141 and Sblock659 which were down-regulated. Functional analysis of these miRNAs by reporter gene assay confirmed a subset of these miRNAs play a potential role in NFL mRNA decrease in ALS. This is the first report describing these novel sequences as miRNAs and their altered expression in ALS spinal cord lysates.

246-A DmGTSF1 is essential for effective retrotransposon silencing by Piwi in Drosophila ovarian somatic cells

Hitoshi Ohtani[1], *Haruhiko Siomi*[1]

[1]Keio University School of Medicine

Drosophila Piwi associates with PIWI-interacting RNAs (piRNAs) and silences transposons to maintain the integrity of the genome in the germline. Previous studies have shown that nuclear localization, but Slicer activity, of Piwi is necessary for Piwi-mediated transposon silencing in ovarian somatic cells (OSCs). However, the underlying mechanism remains unclear. We have screened 100 genes by RNA interference in OSCs and so far identified 12 genes required for transposon silencing in the cells. Of those, we currently focus on DmGTSF1, the *Drosophila* homolog of mouse gametocyte-specific factor 1 (GTSF1), because DmGTSF1 tightly associates with Piwi and is required for Piwi-mediated transposon silencing in OSCs. Mouse GTSF1 has been shown to be essential for transposon silencing in the testes; yet, the molecular function remains unclear. To reveal the role of DmGTSF1 in OSCs, we produced anti-DmGTSF1 monoclonal antibodies. Immunofluorescence on OSCs using the antibodies showed that DmGTSF1 localizes in the nucleus of OSCs. Disruption of DmGTSF1 affected neither the Piwi nuclear localization nor the expression level of piRNAs; however, Piwi-mediated transposon silencing became defective. We are currently analyzing how DmGTSF1 enhances Piwi-mediated nuclear transposon silencing in OSCs, which will be discussed at the meeting.

249-A Withdrawn

252-A A proteomic screen identifies novel regulators of micro-RNA biogenesis

Thomas Treiber[1], *Nora Treiber*[1], *Simone Harlander*[1], *Henning Urlaub*[2], *Gunter Meister*[1]

[1]University of Regensburg; [2]Max Planck Institute of Biophysical Chemistry

MicroRNAs (miRNAs) are a class of small noncoding RNAs that negatively regulates gene expression by destabilization or translational inhibition of mRNAs. miRNAs are encoded in the genome as part of longer primary transcripts and subsequently processed by two nucleolytic events catalyzed by the RNase III enzymes Drosha and Dicer. The expression of miRNAs is tightly regulated, and in many cases deregulation of miRNA abundance has been linked to diseases including cancer. Apart from transcriptional regulation of the primary miRNAs it has become evident that also the maturation steps can be specifically regulated by RNA binding proteins. However, only a small number of posttranscriptional miRNA regulators has been discovered to date. Therefore, we have set up a proteomics-based screen to identify proteins specifically binding to individual miRNA precursors. In a pulldown approach we have screened a set of 72 in vitro transcribed miRNA precorsor sequences against a panel of 11 cancer cell lines and have analyzed the bound proteins by mass spectrometry.

The obtained data revealed about 150 proteins that bind specfically to one or a small number of the tested RNA sequences and thus are candidates for a sequence specific recognition and regulation of individual miRNA precursors. Analysis of the interaction motives suggests that the terminal loop of the hairpin-shaped miRNA precursors serves as main interaction-site in our assay.

In a second screening stage we are currently validating a test set (30) of the candidates in knock down assays to investigate their role in the biogenesis of the bound miRNAs. Initial results indicate that a number of the identified candidates indeed regulates the processing of recognized miRNAs, already now expanding the range of regulatory factors in miRNA biogenesis markedly. Together with additional validation experiments, this project aims to for the first time generate a comprehensive view of posttranscriptional regulation of miRNA biogenesis.

255-A The novel Tetrahymena gene COI12 is crucial for siRNA loading into the Argonaute protein Twi1p

Sophie Wöhrer[1], *Kazufumi Mochizuki*[1]

[1]IMBA - Institute of Molecular Biotechnology

RNA interference pathways have important functions in the regulation of diverse cellular processes. The ciliated protozoan Tetrahymena utilizes a complex RNAi pathway during its sexual reproduction, called conjugation, as a defense mechanism against transposon like sequences. In the somatic genome these sequences are packed into heterochromatin which eventually leads to elimination of the DNA. The correct targeting of these sequences is thought to be achieved via base pairing of ~29nt siRNAs with nascent transcripts.

At the onset of conjugation the nuclear Dicer like enzyme Dcl1p produces the ~29nt siRNAs, so called scan RNAs (scnRNAs). In order to form a functional effector complex these scnRNAs have to be loaded into the Argonaute protein Twi1p. This process takes place in the cytoplasm and is therefore clearly separated from the production of scnRNAs. However, how the loading is regulated is not well understood.

Here we report the characterization of the conjugation-induced gene 12 (*COI12*) which is essential for this loading process. At early stages of conjugation Coi12p localizes to the cytoplasm where scnRNA loading occurs. In the absence of Coi12p immunopurified Twi1p is not in a complex with scnRNAs. As a consequence these small RNAs as well as the Argonaute protein get degraded. Coi12p has a TPR domain, which interacts with Hsp90, and an Fk-binding domain with a yet unclear function. We established in vitro and in vivo systems to manipulate and analyze the loading process at a mechanistic level in more detail and to investigate a possible function of Coi12p and Hsp90p in the loading process. This study could shed more light on the suggested role of the Hsp90 chaperone machinery in the loading process of small RNAs to Argonaute proteins.

RNA Catalysis and Riboswitches

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 258 A – 270 A
Location: Main Hallway & Sanada Foyer

258-A Spliceozymes: Ribozymes that act like Spliceosomes

Zhaleh Amini[1], *Ulrich Müller*[1]

[1]University of California, San Diego

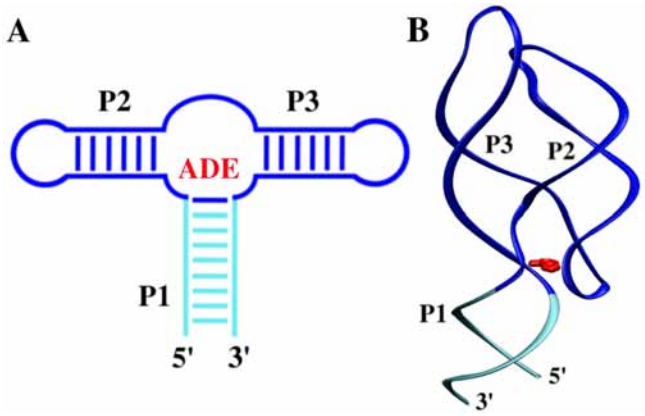
The spliceosome is a large, dynamic RNA-protein particle (RNP) that evolved from a common ancestor with self-splicing group II intron ribozymes. Self-splicing intron ribozymes contain the larger group II intron ribozymes and the smaller group I intron ribozymes. To model the evolution of the spliceosome in the lab we generated a variant of trans-splicing group I intron ribozymes that acts like a spliceosome: The ribozyme variant uses its 5'- and 3'-terminus to recognize two splice sites on a target RNA, catalyzes the removal of the internal sequence, and joins the flanking exons. These 'spliceozymes' appear general with regard to intron length and intron sequence because introns with lengths between 64 and 329 nucleotides were removed, with diverse intron sequences. To test whether these spliceozymes could function like spliceosomes inside a cell we inactivated the mRNA of chloramphenicol acetyl transferase (*CAT*) with an intron and targeted a spliceozyme to remove this intron. In *E.coli* cells, the spliceozyme processed this *CAT* pre-mRNA to functional *CAT* mRNA efficiently enough to mediate *E.coli* growth on medium containing chloramphenicol. We used this growth phenotype in *E.coli* cells to create a model system for the evolution from spliceozymes to spliceosomes: Mutations were introduced into the spliceozyme sequence by mutagenic PCR, and the most efficient spliceozymes were selected by plating the *E.coli* cells on medium containing chloramphenicol. Over multiple cycles of this evolution, the population of spliceozymes accumulated mutations and sampled the protein repertoire of the *E.coli* cells for interactions that benefit the expression of CAT enzyme. The preliminary results of this evolution will be presented at the conference. In summary, our results show that trans-splicing ribozymes can be used to model the first steps in the evolution of the spliceosome.

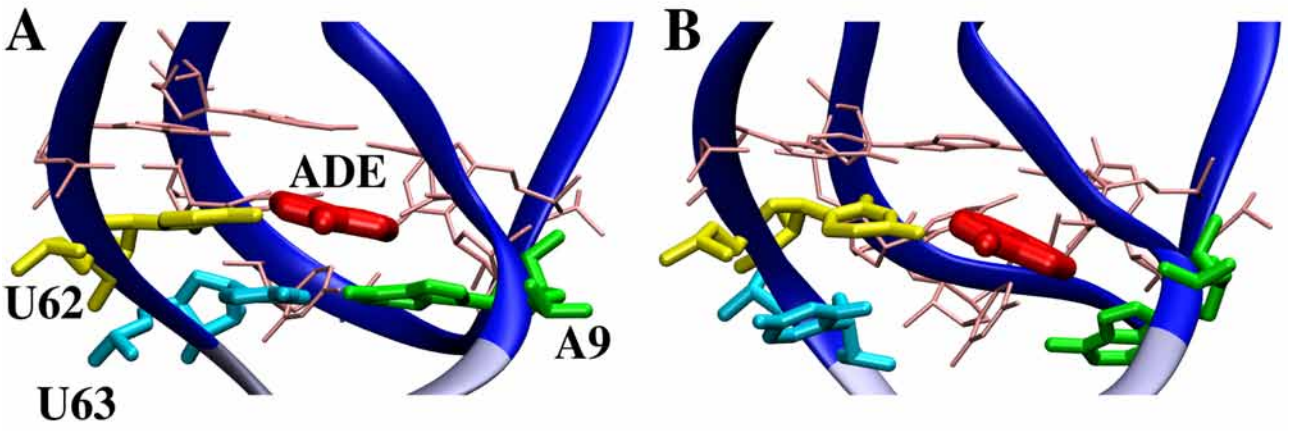
261-A Ligand-induced stabilization of the aptamer terminal helix in the adenine riboswitch

Francesco Di Palma[1], *Francesco Colizzi*[1], *Giovanni Bussi*[1]

[1]**SISSA - Scuola Internazionale Superiore di Studi Avanzati**

Riboswitches are structured mRNA elements that modulate gene expression. They undergo conformational changes triggered by highly-specific interactions with sensed metabolites. Among the structural rearrangements engaged by riboswitches, the forming and melting of the aptamer terminal helix, the so-called P1 stem, is essential for genetic control. The P1 stem, which competes with the repressor, is formed in the ON-state, whereas it is disrupted in the OFF-state. It has been proposed that P1 is stabilized by the ligand and that this could be a common feature in many riboswitch classes. In this context a quantitative estimation of the energetic contributions associated to ligand binding, in particular regarding the role of direct P1-ligand interactions, has not yet been provided and the structural mechanisms by which this conformational change is modulated upon ligand binding mostly remain to be elucidated. State-of-the-art-free-energy methods combined with atomistic simulations can bridge the gap providing an unparalleled perspective on the mechanism and dynamics of the biomolecular process of interest. Here we used steered molecular dynamics simulations to study the thermodynamics of the P1 stem formation in the presence and in the absence of the cognate ligand for the *add* adenine riboswitch. The P1 ligand-dependent stabilization was quantified in terms of free energy (-4.4 kJ/mol) and compared with thermodynamic data from previous dsRNA melting experiments and single-molecule experiments, resulting in nice agreement with them. Our work provides atomistic details and energetic estimates of the process of interest and, altogether with the related experimental works, it suggests a model for the aptamer folding in which direct P1-ligand interactions play a minor role when compared with those related to the ligand-induced aptamer preorganization. Because the structural/functional role of the aptamer terminal helix is a common feature in the "straight junctional" riboswitches, we foresee a wider validity of this model.





264-A Architectural Diversity of PreQ1 Riboswitches

Phillip J. McCown[1], *Jonathan J. Liang*[2], *Ronald R. Breaker*[3]

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Riboswitches are RNA structures that typically bind to small molecules and regulate gene expression. Riboswitches are placed into different classes based on their distinct secondary and tertiary structures and based on their ligands. In previous studies, we determined that a class of riboswitches for pre-queuosine$_1$ (PreQ$_1$) has representatives that conform to two different types based on the presence of unique sequence sub-domains (Roth et al. 2007). Subsequently, we identified a second class of PreQ$_1$ riboswitches that has no structural similarity to the first class (Meyer et al. 2008).

As DNA sequence databases continue to expand in data, additional searches for variants of the PreQ$_1$-I and PreQ$_1$-II riboswitch classes are yielding many more hits. Recently, we uncovered the presence of an additional type for each PreQ$_1$ riboswitch class. For PreQ$_1$-I riboswitches, we determined statistically that all three types of this class are distinct from each other. For PreQ$_1$-II riboswitches, the newly found type is able to discriminate more strongly against binding the immediate biosynthetic precursor of PreQ$_1$, called PreQ$_0$. We have also identified an entirely new class of PreQ$_1$ riboswitches, termed PreQ$_1$-III, which has no structural similarity to the other two classes. Based on this diverse collection of different PreQ$_1$-sensing riboswitches and other studies of PreQ$_1$, it is clear that numerous species of bacterial make extensive use of RNA to directly sense and respond to changing concentrations of this important modified nucleobase.

Roth A, Winkler WC, Regulski EE, Lee BWK, Lim J, Jona I, Barrick JE, Ritwik A, Kim JN, Welz R, Iwata-Reuyl D, Breaker RR. 2007. A riboswitch selective for the queuosine precursor preQ1 contains an unusually small aptamer domain. *Nat. Struct. Mol. Biol.* **14:**308-317.

Meyer MM, Roth A, Chervin SM, Garcia GA, Breaker RR. 2008. Confirmation of a second natural preQ1 aptamer class in Streptococcaceae bacteria. *RNA* **14:**685-695.

267-A A Riboswitch Class for the Bacterial Second Messenger c-di-AMP

James Nelson[1], Narasimhan Sudarsan[3], Kazuhiro Furukawa[2], Zasha Weinberg[3], Joy Wang[2], Ronald Breaker[3]

[1]Department of Chemistry, Yale University, New Haven, CT 06511; [2]Dept. of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06511; [3]Howard Hughes Medical Institute and Dept. of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06511

Riboswitches are small, structured RNA motifs commonly found in the 5'-untranslated regions of certain mRNAs. Each riboswitch class has evolved to selectively recognize a small molecule or ion and change the expression of associated genes. More than two dozen different riboswitch classes have been validated, including RNA motifs that respond to various ligands such as nucleobases, amino acids, coenzymes, second messengers, toxic anions, and other chemicals. The ligand identity of the majority of riboswitches can often be readily deduced by their genomic contexts, but for a few "orphan" riboswitch classes, ligand identification can be challenging. Some orphan riboswitch candidates are associated with many different genes, or with genes encoding proteins of unknown functions, which make ligand identification difficult.

A metabolite-binding riboswitch candidate called *ydaO* was discovered in bacteria nearly a decade ago, but its ligand has remained unknown. This predicted riboswitch class ranks among the top ten most common metabolite-sensing RNAs known, and it is associated with numerous genes for cell wall metabolism, osmotic stress response, and sporulation. We report that members of this noncoding RNA class selectively respond to the recently-discovered bacterial second messenger cyclic di-adenosine monophosphate (c-di-AMP). A representative RNA from *Bacillus subtilis* binds c-di-AMP with an affinity that is more than one million fold higher than for the c-di-AMP precursor ATP and for the bacterial second messenger c-di-GMP. Our findings resolve the mystery regarding the ligand for this extremely common riboswitch class, and expose a major portion of the super-regulon of genes that are controlled by the widespread bacterial second messenger c-di-AMP.

270-A A pinch model for activation of HDV-like ribozymes

Chiu-Ho Webb[1], *Andrej Luptak*[1]
[1]University of California-Irvine

The HDV family of ribozymes are widely distributed in nature and their biological roles include processing of viral and retrotransposon transcripts and translation initiation of retrotransposon mRNAs. These ribozymes are characterized by their double-pseudoknot secondary structure. Many retrotransposon-associated ribozymes also contain large insertions predicted to form stable structures. We show that these peripheral domains activate the ribozymes by "pinching" two core helices together. A *trans*-cleaving construct derived from the *Drosophila* R2 ribozymes consisting of a ribozyme subunit and a long substrate strand that spans the core and peripheral domains exhibits a large activation barrier, supporting the pinch model. Our data point to a simple mechanism of activity modulation in HDV-like ribozymes (seeFig. 1).

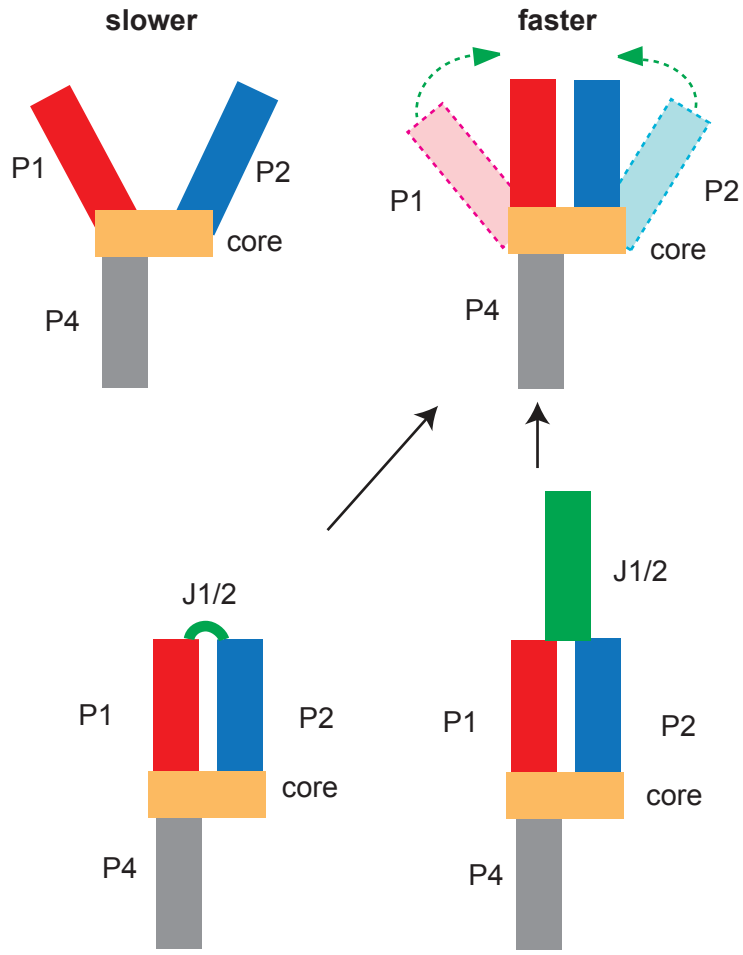


Figure 1

RNA structure and folding

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 273 A – 297 A
Location: Main Hallway & Sanada Foyer

273-A The solution structure of Tetrahymena telomerase p65-RNA(TER)-TERT RBD determined by SAXS reveals contacts critical for particle stability

Andrea Berman[1], Anne Gooding[4], Robert Rambo[2], John Tainer[3], Thomas Cech[4]

[1]University of Colorado; [2]Lawrence Berkeley National Laboratory; [3]Lawrence Berkeley National Laboratory, Physical Bioscience Division; [4]University of Colorado, Boulder and HHMI

The *Tetrahymena* telomerase ribonucleoprotein particle catalyzes telomeric DNA synthesis at the end of eukaryotic chromosomes. Although it minimally contains a reverse transcriptase protein subunit (TERT) and a template-containing RNA (TER), the biogenesis of the enzyme complex requires the protein p65[1,2], which binds TER and induces a conformational change that facilitates the binding of TERT[3-5]. We used small-angle x-ray scattering (SAXS) to determine the low-resolution solution structures of p65 alone, a portion of the TER alone, p65 bound to TER and p65-TER in complex with the RNA binding domain (RBD) of TERT. The SAXS analysis has allowed us to confidently distinguish the RNA from the protein in the scattering density. This has significantly aided in modeling the SAXS envelopes with published crystal and NMR structures of the RNA and protein components, allowing us to understand the global arrangement of the minimal holoenzyme[2,]. Our structure reveals an elongated arrangement of the domains of p65. The observation that p65 contacts both TER and TERT supports the hypothesis that p65 serves as a molecular buttress that stabilizes both the TER and TERT in their catalytically active conformations[6]. Comparison of SAXS models of p65 complexed with constructs of TER containing and lacking the pseudoknot indicates that the pseudoknot is required for the proper folding of TER. These data offer insight into how the three molecules in the minimal complex interact with each other to make a functional telomerase and provide additional evidence for why the pseudoknot is essential in catalysis[7].

1. Prathapam, R., Witkin, K.L., O'Connor, C.M. & Collins, K. A telomerase holoenzyme protein enhances telomerase RNA assembly with telomerase RNA assembly with telomerase reverse transcriptase. *Nat Struct Mol Biol* **12**, 252-7 (2005).

2. Witkin, K.L. & Collins, K. Holoenzyme proteins required for the physiological assembly and activity of telomerase. *Genes Dev* **18**, 1107-18 (2004).

3. O'Connor, C.M. & Collins, K. A novel RNA binding domain in tetrahymena telomerase p65 initiates hierarchical assembly of telomerase holoenzyme. *Mol Cell Biol* **26**, 2029-36 (2006).

4. Stone, M.D. et al. Stepwise protein-mediated RNA folding directs assembly of telomerase ribonucleoprotein. *Nature* **446**, 458-61 (2007).

5. Singh, M. et al. Structural basis for telomerase RNA recognition and RNP assembly by the holoenzyme La family protein p65. *Mol Cell* **47**, 16-26 (2012).

6. Berman, A.J., Gooding, A.R. & Cech, T.R. Tetrahymena telomerase protein p65 induces conformational changes throughout telomerase RNA (TER) and rescues telomerase reverse transcriptase and TER assembly mutants. *Mol Cell Biol* **30**, 4965-76 (2010).

7. Tzfati, Y., Knight, Z., Roy, J. & Blackburn, E.H. A novel pseudoknot element is essential for the action of a yeast telomerase. *Genes Dev* **17**, 1779-88 (2003

276-A Symmetry and asymmetry in the unwinding of nucleic acids

Francesco Colizzi[1], *Yaakov Levy*[2], *Giovanni Bussi*[1]

[1]SISSA - Scuola Internazionale Superiore di Studi Avanzati; [2]Department of Structural Biology, Weizmann Institute of Science, Rehovot, Israel

The forming and melting of complementary base pairs in RNA and DNA duplexes are conformational transitions required to accomplish a plethora of biological functions. Using fully atomistic simulations we have shown that RNA unwinding occurs by a stepwise process in which the probability of unbinding of the base on the 5′ strand is significantly higher than that on the 3′ strand [Colizzi and Bussi JACS, 2012]. The asymmetry in the RNA unwinding dynamics is compliant with the mechanism of helicase activity shown by prototypical DEx(H/D) RNA helicases and could allow deciphering the basis of the evolutionary pressure responsible for the unwinding mechanism catalyzed by RNA-duplex processing enzymes. In this spirit and from a broader standpoint, here we use a topology-based coarse-grain model to compare and characterize the mechanism of unwinding for both DNA and RNA. The (a)symmetric behavior of the 3′- and 5′-strand could be related to the (bi)directionality observed in molecular machineries processing nucleic acids.

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

Neena Grover[1]

[1]Department of Chemistry and Biochemistry, Colorado College

Bulges are found in most small and large RNA. The presence of a bulge alters the A-form helical structure and allows the formation of many non-canonical base pairs. Thus unique conformations of the bulge region become sites for recognition and interactions with other biomolecules. We are comparing small bulged RNA to determine sequence and ionic conditions in which a particular non-canonical base pair forms and its contribution to RNA stability. For our studies, RNA constructs containing small symmetric or asymmetric bulges are derived from functionally interesting and well-characterized RNA. Thermodynamic data on small RNA constructs is collected in 1 M KCl buffers where charge-charge interactions are expected to be fully satisfied, based on polyelectrolyte theory of ion condensation. Comparative analysis of $?G^{o}_{37}$ data on multiple RNA constructs in 1 M KCl is utilized to measure the contribution (if any) of non-canonical base pairs. Measurements are also performed in 0-10 mM magnesium ions to determine the contributions of magnesium ions in stabilizing these constructs. The penalty for breaking an A-form helix in RNA can be several kcal/mol. Formation of strong non-canonical base pairs in the bulge, such as C+•C, provides significant stabilization and minimizes the cost of breaking the helical structure. In the presence of 10 mM of magnesium ions, RNA constructs are equally or more stable ($?G^{o}_{37}$) than in 1 M KCl. An additional 2-4 kcal/mol of free energy is available in 10 mM magnesium buffer for constructs in which magnesium interactions are significant. A-form helical constructs also show stabilization in magnesium ions depending on the sequence. Bulged containing DNA constructs examined were not additionally stabilized by magnesium ions over 1 M KCl. Thermodynamic analysis provides accurate and straightforward method to measure the strength of non-canonical base pairs and ion-binding in nucleic acids. This work is supported by NSF Grant MCB-0950582.

282-A A predictive value optimizing the evaluation of biological RNA G-quadruplexes formation

Rachel Jodoin[1], Jean-Denis Beaudoin[1], Jean-Pierre Perreault[1], Martin Bisaillon[1]

[1]Université de Sherbrooke, Sherbrooke, Quebec, Canada

RNA G-quadruplexes are non-canonical structures involved in many important cellular processes such as translation regulation and mRNA polyadenylation. They are composed of stacks of at least two G-quartets, a co-planar arrangement of four guanines linked together by Hoogsteen base-pairs and stabilized by monovalent cations, usually K^+. Sequences agreeing to the algorithm G_x-N_{1-7}-G_x-N_{1-7}-G_x-N_{1-7}-G_x (where x = 3, N=A,U,G,C) are considered as potential candidates for G-quadruplexes (PG4). However, many sequences identified as forming G-quadruplexes with known biological functions do not correspond to this algorithm. Moreover, this algorithm led to the identification of a number of false positives, hence there is a need to consider other factors than the PG4 sequence only. For example, we previously reported that many false positive PG4s do not form G-quadruplexes because of the presence of multiple tracks of cytosines (C) in their genomic context competing to form Watson-Crick structures [1]. In order to determine the extent of the genomic context's influence on G-quadruplex formation, we selected 12 predicted PG4s found in the 5'- and 3'-untranslated region (UTR) of human mRNAs. *In vitro,* we have assessed the adoption of the G-quadruplex structure for the same PG4 with either 15 or 50 nucleotides of their upstream and downstream genomic context by in-line probing. We then sought to find a predictive value representative of the competitiveness of the genomic context. Several analyses have allowed the development of a new scoring system for the prediction of PG4 considering the sequence in the vicinity. This new system could be used in combination with the standard algorithm as a scoring-system to optimize RNA G-quadruplexes prediction.

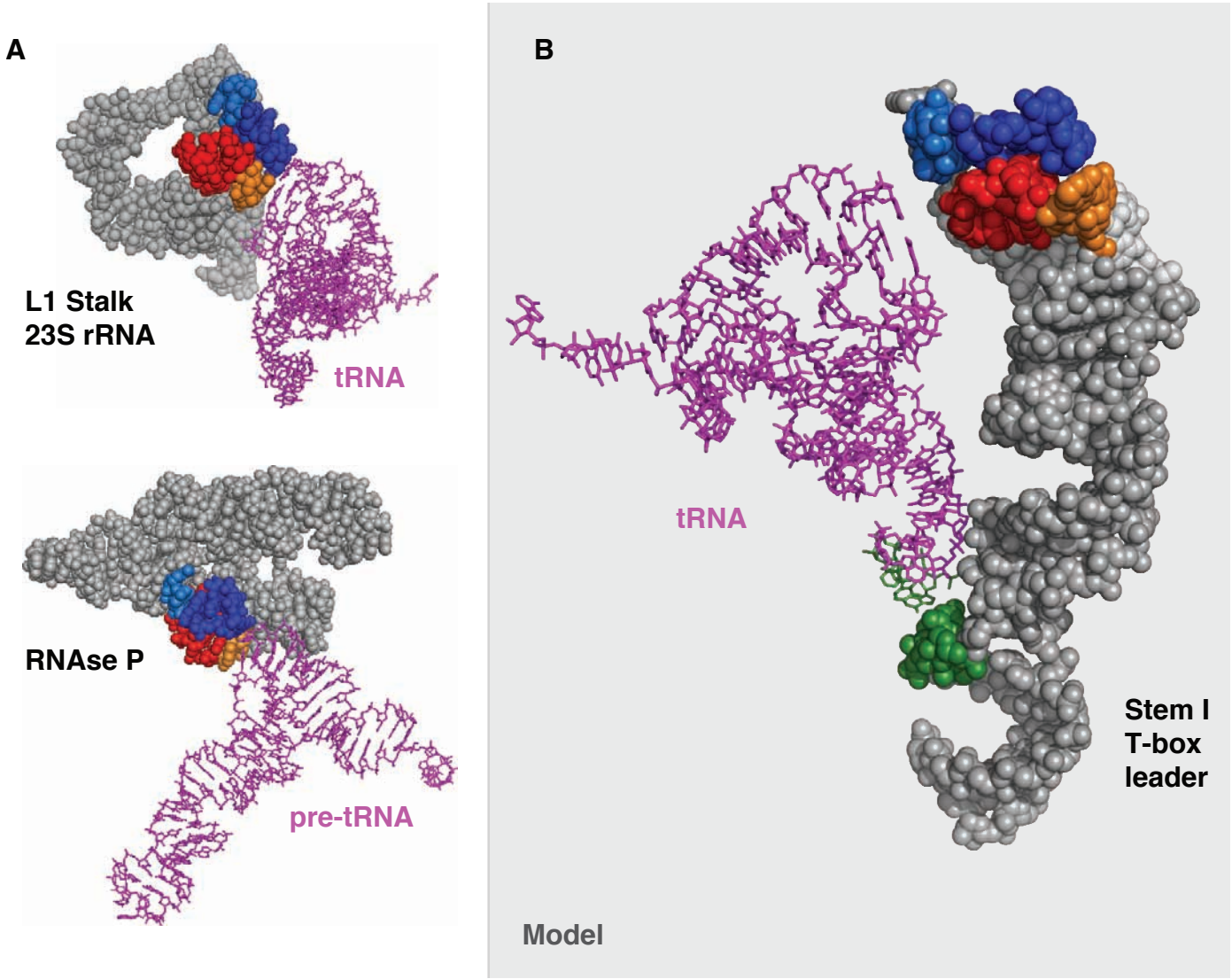
1. 1. Beaudoin, JD. and Perreault, JP. (2010) *Nucleic Acids Research*, **38**, 7022-7036

285-A A Universal RNA Structural Motif Docking the Elbow of tRNA in the Ribosome, RNAse P and T-box Leaders

Jean Lehmann[1], *Fabrice Jossinet*[2], *Daniel Gautheret*[1]

[1]Institute of Genetics and Microbiology, University Paris-Sud, France; [2]Institute of Molecular and Cellular Biology - CNRS, University of Strasbourg, France

The structure and function of conserved motifs constituting the apex of Stem I in T-box mRNA leaders are investigated. We point out that this apex shares striking similarities with the L1 Stalk (helices 76-78) of the ribosome. A sequence and structural analysis of both elements shows that, similarly to the head of the L1 Stalk, the function of the apex of Stem I lies in the docking of tRNA through a stacking interaction with the conserved G19:C56 base pair platform. The inferred structure in the apex of Stem I consists of a module of two T-loops bound together head-to-tail, a module which is also present in the head of the L1 Stalk, but went unnoticed. Supporting the analysis, we show that a highly conserved structure in RNAse P formerly described as the J11/12-J12/11 module, which is precisely known to bind the elbow of tRNA, constitutes a third instance of this T-loop module. A structural analysis explains why six nucleotides constituting the core of this module are highly invariant among all three types of RNA. Our finding that major RNA partners of tRNA bind the elbow with a same RNA structure (Figure 1) suggests an explanation for the origin of the tRNA L-shape.



Legend of Figure 1:

Crystallographically established (a) and model (b) of tRNA – RNA binding in which a stacking interaction between the G19:C56 base pair platform defining the elbow of a tRNA and a platform of a head-to-tail double T-loop module is involved. **(a)** L1 Stalk – tRNA (pdb *1VSA*) and RNAse P – pre-tRNA (pdb *3Q1Q*) interactions. Only a fragment of RNAse P is shown. **(b)** Model of *B. subtilis ProI* Stem I – tRNA interaction.

288-A Structural characterization of the yeast telomerase RNA core by SHAPE

Rachel O. Niederer[1], *David C. Zappulla*[1]

[1]**Johns Hopkins University**

Telomerase is a ribonucleoprotein (RNP) that catalyzes the addition of telomere repeats to the ends of linear eukaryotic chromosomes. The core enzyme consists of telomerase reverse transcriptase (TERT) and telomerase RNA (TR). Our aim is to investigate the overall architecture of *S. cerevisiae* telomerase RNA (TLC1) to gain insights into the relationship between RNA structure and telomerase function. The large size of TLC1 (1157 nts) makes structural characterization difficult. To overcome this obstacle, we took advantage of an active 170-nt yeast telomerase RNA, Micro-T. This catalytic core RNA contains the conserved structural elements, including the 16-nt template sequence that is reverse transcribed, a catalytically important pseudoknot, and a poorly characterized protein-binding region. We find that Micro-T adopts fewer conformations compared to TLC1 *in vitro* by native gel electrophoresis, suggesting it should be more amenable to structural studies. We investigated the structure of this RNA using Selective 2'-Hydroxyl Acylation analyzed by Primer Extension (SHAPE), which interrogates local flexibility at each nucleotide position in a given RNA. Here we report the first application of SHAPE chemistry on yeast telomerase RNA. Our Micro-T SHAPE analysis supports the current secondary structure model for the TLC1 core. The template nucleotides and adjacent single-stranded region are highly SHAPE reactive, suggesting the RNA is in a biologically relevant conformation. Furthermore, our data indicate that the yeast pseudoknot is able to form in the absence of TERT. This is in contrast to the in-vitro folding of protein-free *Tetrahymena thermophila* TR, which does not form a pseudoknot or maintain an unpaired template. This suggests the intrinsic stability of the yeast pseudoknot is more similar to that of human TR. SHAPE will allow further examination of structure-function relationships in the telomerase RNP. For instance, in human telomerase evidence suggests that physical flexibility resulting from an unusual 5-nucleotide bulge within the pseudoknot is important for catalysis. We propose physical flexibility within the pseudoknot is a prominent feature of all telomerase RNAs. Although yeast does not contain a bulge at the corresponding position, it has an unusually long unpaired region, which we find to be reactive by SHAPE. We are now testing for conformational changes in telomerase RNA the presence of TERT. Results from these studies will help determine how yeast telomerase RNA and TERT interact and coordinate telomeric DNA synthesis.

291-A The influence of metal ions on the structure of the CPEB3 ribozyme's P4 region

Miriam Skilandat[1], *Magdalena Rowinska-Zyrek*[1], *Roland K. O. Sigel*[1]

[1]University of Zurich, Institute of Inorganic Chemistry, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

The CPEB3 ribozyme is one of two small ribozymes that have been discovered in mammalian genomes (Salehi-Ashtiani et al. 2006; Teixeira et al. 2004). This small self-cleaving RNA is exclusively found in mammals with a highly conserved sequence and resides on an intron of the *cpeb3* gene (Salehi-Ashtiani et al. 2006). The function of the CPEB3 ribozyme is yet unknown, but its secondary structure and cleavage mechanism have been proposed to be highly similar to the one of the Hepatitis Delta Virus ribozyme (Salehi-Ashtiani et al. 2006), which uses Mg^{2+} ions for cleavage (Nakano et al. 2001).

As a first step in the investigation of the 3D structure of the CPEB3 ribozyme and of its interaction with metal ions, we have solved the solution structure of the P4 region of the ribozyme and investigated its metal-ion binding sites using NMR-spectroscopy. This small hairpin is closed by a UGGU tetraloop, which is stabilized by base stacking interactions and an internal hydrogen bond and which contains one inner-sphere Mg^{2+}-binding site. The tetraloop's structural features are very similar to the ones previously described for an AGUU tetraloop being an RNAse III recognition motif in yeast (Wu et al. 2001). A second Mg^{2+}-binding site is contained in the stem of P4, sequentially close to the proposed ribozyme active site. Comparison of the NMR spectra of the full-length CPEB3 ribozyme and the free P4 hairpin in the absence and in the presence of Mg^{2+} indicates that the structure and the Mg^{2+}-binding sites of the isolated P4 are identical or very similar to the ones found in the structural context of the full-length ribozyme, thus providing first information on the CPEB3 ribozyme's fold and metal-ion interaction sites.

Financial support by the Swiss National Science Foundation, the European Research Council and the University of Zurich is gratefully acknowledged.

Nakano S, Proctor DJ, Bevilacqua PC (2001) Mechanistic Characterization of the HDV Genomic Ribozyme: Assessing the Catalytic and Structural Contributions of Divalent Metal Ions within a Multichannel Reaction Mechanism. *Biochemistry* **40:** 12022-12038

Salehi-Ashtiani K, Lupták A, Litovchick A, Szostak JW (2006) A Genomewide Search for Ribozymes Reveals an HDV-Like Sequence in the Human CPEB3 Gene. *Science* **313:** 1788-1792

Teixeira A, Tahiri-Alaoui A, West S, Thomas B, Ramadass A, Martianov I, Dye M, James W, Proudfoot NJ, Akoulitchev A (2004) Autocatalytic RNA cleavage in the human ß-globin pre-mRNA promotes transcription termination. *Nature* **432:** 526-530

Wu H, Yang PK, Butcher SE, Kang S, Chanfreau G, Feigon J (2001) A novel family of RNA tetraloop structure forms the recognition site for *Saccharomyces cerevisia*e RNase III. *EMBO Journal* **20:** 7240-7249

294-A Automated identification of RNA 3D modules with discriminative power in RNA structural alignments.

Corinna Theis[1], Christian Hoener zu Siederdissen[2], Ivo L. Hofacker[2], Jan Gorodkin[1]

[1]University of Copenhagen; [2]University of Vienna

The particular three-dimensional architecture, which enables structured RNAs to accomplish a variety of functions, is established by local or remote tertiary interactions. A set of such tertiary interactions enclosed by canonical base pairs is called an RNA 3D module. The steady growth of experimentally determined atomic resolution RNA structures (X-ray or NMR) have revealed different types of RNA 3D modules, such as kink-turns, C-loops and G-bulges. Even though they in generally only consist of a few nucleotides, they play a major role in mediating protein and ligand docking, directing the folding process or stabilizing the structural conformation of an RNA molecule. The RNA 3D modules have shown up over numerous structures as recurring building blocks, which often are conserved throughout all kingdoms of life. Thus, it is a general interest to match RNA 3D modules known from one molecule to another for which the 3D structure is not known or the RNA sequences are only partially known. Recent strategies in RNA structure prediction include to 'fill the gap' in 2D RNA structure by including 3D information into the structure prediction, such as probing data. The assignment of such 3D information to 2D structures can improve structure prediction, limits the number of false positives, assign functions to unknown molecules, help to find new modules as well as classifying transcripts as ncRNAs.

Here, we address the usage of 3D information, by merging RNA 3D modules in existing 2D structural alignments of RNA. To meet this challenge we have created a pipeline, called metaRNAmodules, which completely automates extracting of known and unknown modules from the FR3D database and subsequently map them to Rfam structural RNA alignments to obtain comparative information. Subsequently, the modules are turned into graph-based statistical models (using Bayesian networks) for the RMDetect program, which allows for test of their discriminative power using real and shuffled Rfam alignments. An initial extraction of 15290 RNA 3D modules in all PDB files results in 977 modules with clear discriminative power. Many of these modules describe only minor variants of each other. Indeed, mapping of the modules onto Rfam families results in 28 unique locations in 10 different families. Our pipeline is available as source code along with the generated RNA 3D modules at http://rth.dk/resources/mrm.

297-A How do platinum drugs interact with RNA?

Marianthi Zampakou[1], *Daniela Donghi*[1]

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We are currently studying the influence of platinum drugs on RNA structure, with a special focus on the anticancer drug cisplatin and its derivatives. Such drugs are generally believed to mainly target DNA by preferentially binding to the N7 atoms of two neighbouring guanines.[1] Nevertheless, additional potential targets are likely to exist, including RNA.[2]

In fact, cisplatin was shown to inhibit RNA-dependent processes.[2] Moreover, in the last decades several biochemical studies have been reported confirming the interaction of platinum drugs with RNA.[2,3] However, still very little is known on RNA structural changes upon platinum binding. The presence in RNA of loops, bulges and junctions, eventually important for tertiary contacts, possibly opens up a multifaceted scenario for RNA-platinum drug interaction.

The goal of our project is to obtain structural information on how platinum drugs interact with RNA, to evaluate actual structural preferences in the binding. For this purpose, we use a 27nt RNA hairpin model system derived from the yeast mitochondrial group II intron *Sc*.ai5G.[4] The structure of this construct in solution is known,[5] and it contains secondary structure motifs common in RNAs.[6] Different platination agents are used, including cisplatin and oxaliplatin. The platination products are characterised by several techniques, like mass spectrometry, UV spectroscopy and gel mobility shift assays. We are currently optimising large scale platination conditions to obtain higher amount of pure platinated sample to be used for structure evaluation by NMR spectroscopy.

Acknowledgements. Financial support by the Swiss National Science Foundation (*Ambizione* fellowship PZ00P2_136726 to DD), by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged.

[1] Aldeerden RA, Hall MD, Hambley TW. 2006. *J. Chem. Educ.*, **83:** 728-734.

[2] Chapman EG, Hostetter AA, Osborn MF, Miller AL, DeRose VJ. 2011. *Met. Ions Life Sci.*, **9:** 347-377.

[3] See for example Papsai P, Snygg AS, Aldag J, Elmroth SKC. 2008. *Dalton Trans.*, 5225-5234.

[4] Sigel RKO. 2005. *Eur. J. Inorg. Chem.*, 2281-2292.

[5] Donghi D, Pechlaner M, Sigel RKO, *unpublished*.

[6] Butcher SE, Pyle AM. 2011. *Acc. Chem. Res.*, **44:** 1302-1311.

RNA chemistry

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 300 A – 306 A
Location: Main Hallway & Sanada Foyer

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300 A **Inhibition of Bcl-2 using photo-crosslinking antisense oligonucleotides**
303 A **Suppression of miR-29a maturation by ligand binding**
306 A **Versatile phosphoramidation reactions for effective labeling and conjugation of nucleic acids**

300-A Inhibition of Bcl-2 using photo-crosslinking antisense oligonucleotides

Akio Kobori[1], *Yuko Nagae*[1], *Asako Yamayoshi*[1], *Akira Murakami*[1]

[1]Kyoto Institute of Technology

Oligonucleotide analogues forming covalent bonds with complementary nucleotides in a sequence-specific manner under physiological conditions are of potential clinical and biological interest. In particular, photoresponsive oligonucleotide analogues which cross-link with complementary nucleotides using photo-irradiation as a trigger of the reaction have been developed to investigate and control gene functions without damaging living systems. These oligonucleotide analogues have photoresponsive moieties introduced on a site which does not affect duplex or triplex formation. The introduced photoresponsive moieties, which are non-reactive or less reactive toward bio-molecules in their original form, generate highly reactive species after photo-irradiation. We have reported that oligonucleotides having a psplaren derivative (Ps) at the 2'-*O* hydroxy group of adenosine (2'-Ps-eom) recognize one base difference in the target sequences under clinically relevant conditions. Using 2'-Ps-eom, we successfully achieved the inhibition of K-*ras*-immortalized cell proliferation (K12V) but not of Vco cells that contain the wild-type K-*ras* gene. Considering the potential benefits of photoresponsive cross-linking reagent-nucleotide conjugates, it is important to develop new photoresponsive groups that could give high yields and exhibit specific reactive characteristics to target nucleobases located in specific positions in the target sequence. In this study, we newly synthesized photo-cross-linking ODNs having a photoresponsive a-chloroaldehyde (PCA) group at the 5'-end of the ODN. The PCA group was comprised of an a-chloro bis(2-nitrobenzyl)acetal group, which was converted to an a-chloroaldehyde group after 1 min of UV irradiation. Photo-cross-linking studies revealed that the oligonucleotide conjugates underwent sequence-selective cross-linking to target nucleotides in a time-dependent manner under physiological conditions.

303-A Suppression of miR-29a maturation by ligand binding

Takahiro Otabe[1], *Asako Murata*[1], *Fumie Takei*[1], *Kazuhiko Nakatani*[1]

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In recent years, the discovery of functional non-coding RNA (ncRNA) has raised the interest to modulate the function of ncRNA molecules. Small molecules that bind to specific secondary structural motifs in RNA will provide valuable tools for modulating and studying RNA function.

We have developed a series of naphthyridine derivatives that can bind to single nucleotide bulges in DNA duplexes. Among these derivatives, *N,N*-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine (DANP) was found to bind to cytosine and guanine bulges in RNA duplexes. The stabilizing effect of DANP on the complex with bulged RNA duplexes, however, was rather smaller than that observed for bulged DNA duplexes. The results of molecular modeling of RNA-DANP complex indicate that stacking interaction of DANP with the neighboring bases in the RNA duplex would be insufficient compared with that of DNA-DANP complex, which explain the reduced ability of DANP for stabilizing the RNA-DANP complex. We therefore designed BzDANP that has the same hydrogen-bonding surface as that of DANP but has an expanded aromatic plane in order to examine the effect of the molecular size and the π-stacking with the neighboring base pairs on binding affinity with RNA duplexes.

In this study, we describe the synthesis and the evaluation of binding capability of BzDANP to RNA duplexes containing a single nucleotide bulge. BzDANP was synthesized from 2-amino-7-bromo-isoquinoline as a starting material in five steps. The binding ability of BzDANP to RNA duplexes containing a single bulge was evaluated by thermal melting studies. A significant increase in melting temperature with RNA duplexes containing a single bulge was observed in presence of the BzDANP, suggesting the increased stabilizing effect of BzDANP on bulged RNA duplex relative to DANP. Next we explored a potential inhibitory of BzDANP against the dicing reaction of precursor-miRNA (pre-miRNA) upon binding to its secondary structures. Pre-miR-29a has a cytosine bulge near the putative cleavage sites by Dicer, so we expected that the binding of BzDANP to the bulge would interfere the dicing reaction of pre-miR-29a. Chemically synthesized pre-miR-29a was digested with recombinant human Dicer and the reaction products were analyzed by denaturing PAGE. Dicer digested pre-miR-29a to produce multiple bands on the gel when stained with SYBER GOLD. The rapidly migrating bands were obviously mature miR-29a and star miR-29a, and the others are likely intermediates where anick was made at either strand of the stem. The band intensity of both mature miR-29a and intermediates were decreased with increasing concentration of BzDANP, suggesting the inhibitory effect of BzDANP on pre-miR-29a processing.

306-A Versatile phosphoramidation reactions for effective labeling and conjugation of nucleic acids

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Chemical conjugations of post-synthetic nucleic acids with macromolecules or smaller tag molecules are common approaches to study nucleic acids in chemistry and biology, and to exploit nucleic acids for medical applications. For example, conjugations of nucleic acids such as oligonucleotide siRNA and miRNA with peptides to acquire peptide-oligonucleotide conjugates (POCs) is especially useful to circumvent cell delivery and specificity problems of RNA as therapeutic agents. Recently, my laboratory has developed an aqueous-phase two-step nucleic acid phosphoramidation method for effective POC synthesis (Wang *et al.*, 2010, *Bioconjugate Chem.* **21***,* 1642–1655; Wang *et al.*, 2012, *Bioconjugate Chem*., **23**, 2417-2433). Here we discuss the chemistry of nucleic acid phosphoramidation reactions and applications of the reactions for post-synthetic RNA conjugation and labeling essential to chemical and biological studies of RNA. When coupling with bio-orthogonal reactions such as click reactions, phosphoramidation reactions could be an even more powerful approach for nucleic acid conjugation and labeling. We are expecting that phosphoramidation reactions will have broader applications in conjugation and labeling of nucleic acids useful in fundamental research and in clinic.

Therapeutic RNAs

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 309 A – 315 A
Location: Main Hallway & Sanada Foyer

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309 A **Messenger RNA as a novel therapeutic approach**

312 A **RNA aptamer C3 is a non-ATP site inhibitor of the MAP kinase ERK2**

315 A **RNA-i THERAPY FOR FRONTO TEMPORAL DEMENTIA AND PARKINSONISM LINKED TO CHROMOSOME 17**

309-A Messenger RNA as a novel therapeutic approach

Antonin de Fougerolles[1]

[1]Moderna Therapeutics

Moderna is pioneering messenger RNA Therapeutics™, a novel biotherapeutic modality with the unprecedented capability of stimulating the body's natural ability to produce therapeutic proteins. If successful in human clinical trials, this advance will usher in the first entirely new way of making therapeutic proteins since the development of recombinant technology more than 30 years ago, with dramatic implications for both patients and industry. Moderna is using this approach to develop first-ever treatments for a wide range of diseases that cannot be addressed today using existing technologies, and to drastically reduce the time and expense associated with creating therapeutic proteins using recombinant technologies.

Any protein biologic, antibody or vaccine can be rapidly made using mRNA. The technology is "cell-free", and alleviates the need for laborious and expensive *in vitro* tissue culture or protein manufacture and purification. A new messenger RNA can be developed very quickly, in only a matter of hours (when producing a known mRNA) to weeks (when going from concept to new drug), thereby saving precious time and R&D resources. Moderna has conducted proof of concept studies in numerous preclinical models, including non-human primates and has demonstrated the ability to induce *in vivo* production of dozens of intracellular and secreted therapeutic proteins through intramuscular, subcutaneous or intravenous administration. Pre-clinical programs have been established focused on four key therapeutic areas: oncology supportive care, inherited genetic disorders, hemophilia and diabetes. Results will be discussed.

312-A RNA aptamer C3 is a non-ATP site inhibitor of the MAP kinase ERK2

Sabine Lennarz[1], *Günter Mayer*[2]

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Mitogen-activated protein kinases (MAPK) play a central role during signal transduction in eukaryotes. The ERK (extracellular signal-regulated kinase) MAP kinase pathway is of profound importance for cellular processes, such as proliferation and differentiation.

Since aberrant ERK signaling is a potent driver of oncogenesis in most human cancers, much attention has been focused on the development of inhibitors that target this pathway. The major class of available MAP kinase inhibitors is represented by so-called ATP analogues that inhibit phosphorylation of substrates and downstream signaling by competing with ATP in cells. Unfortunately, ATP-analogues often display poor specificity because the ATP-site is highly conserved amongst kinases. Thus, there is a high demand for the development of novel MAP kinase signaling inhibitors that enable selective inhibition by targeting kinase domains and individual sub-domains specifically.

Besides low-molecular weight ligands and antibodies, aptamers represent a promising class of molecules that can be applied extra- and intracellularly as high affine and specific inhibitors of biomolecules. Aptamers are short, single stranded nucleic acids that fold into a well-defined three-dimensional structure upon which they bind to a specific target molecule.

By performing an *in vitro* selection approach, we identified a RNA aptamer, namely C3, which bound to the MAP kinases ERK1 and ERK2 with high affinity and, furthermore, revealed high specificity, since no binding to other members of the MAP kinase superfamily or a panel of 10 other kinases was detected. More importantly, we were able to show that this aptamer interacts with ERK2 in an ATP-independent manner.

Aptamer C3 was identified as an inhibitor with an alternative and novel mode of action in respect of kinase activity perturbation. We could detect that the putative binding site of C3 on ERK2 includes the so-called "F-recruitment site" docking domain. By targeting a domain on ERK2 that is involved in regulating interactions with activators and substrate proteins, we were able to potently inhibit the activation of ERK2 by its activating kinase MEK1 *in vitro*. Future studies will be performed to investigate the aptamers potential to selectively block interactions of ERK2 with substrates.

Taken together, our data indicates that aptamer C3 binds to a physiologically relevant side on ERK and therefore could pave the way for the development of alternative acting and selective kinase inhibitors.

315-A RNA-i THERAPY FOR FRONTO TEMPORAL DEMENTIA AND PARKINSONISM LINKED TO CHROMOSOME 17

Kavitha Siva[1], Giuseppina Covello[2], Michela Alessandra Denti[2]

[1]PhD student at Centre for Integrative Biology (CIBIO), University of Trento,Italy; [2]Centre for Integrative Biology (CIBIO), University of Trento, Italy

Abnormalities of microtubule associated protein tau have been shown to be linked to pathogenesis of neurodegenerative disease collectively termed as "Tauopathies. Gene mutations in tau lead to perturbation of gene splicing and tau fibrillization leading to formation of tau aggregates. A missense mutation in exon 10 at codon 279, results in an asparagine to lysine substitution (N279K). This impinges alternative splicing of exon 10 of the tau mRNA, and amends the normal ratio of 4Rtau/3Rtau,This in turn leads to an increased expression of 4R tau causing agglomeration of tau proteins.

RNA interference has proven to be an efficient strategy for silencing mutant alleles of dominant disease genes as in Alzheimer's disease, Machado-Joseph disease, Spinocerebellar ataxia type 3 and tau mutation (V337M) that causes fronto-temporal dementia.

This project explores the feasibility of a siRNA-based gene therapy to enable post-transcriptional gene silencing of Exon10 in FTDP-17. A panel of siRNAs targeting Tau exon 10 have been synthesised, which will be further tested upon PC12 cells and Ren cells. The outcome of RNA interference will be tested by both RT-PCR and western blot analysis. Based on these results, the small interfering RNA sequences will be embedded in siRNA expressing vector (psiUx) relying on strong and ubiquitous pol II dependent promoter of human U1 small nuclear RNA (U1 snRNA) gene. Allele specific silencing effects of these constructs will be monitored and analysed in PC12 cells. The effect on human tau pre-mRNA, will be monitored via a mini-gene reporter system, recapitulating to a large extent the behaviour of exon 10 in the context of tau gene.

Further work will be directed to test the therapeutic efficacy of the AAV-vectored sh-RNAs in the animal model of FTDP-17 (T-279 mouse) which recapitulates the disease from a histopathological and behavioural point of view. Preliminary data on the behavioural analysis will be presented.

tRNA, snRNA, snoRNA, rRNA

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 318 A – 333 A, 722 A
Location: Main Hallway & Sanada Foyer

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318 A **Mitochondrial poly(A) polymerase is involved in tRNA editing**

321 A **Structure and Kinetic Mechanism of Protein-only RNase P from A. thaliana**

324 A **Identification of proteins co-purifying with the yeast RNA exosome and their effect on the complex stabilization**

327 A **Absence of a Universal Element for tRNAHis Identity in Eucarya**

330 A **A Protease that Cleaves the C-terminal Domain of the RtcB-type RNA Ligase in the Hyperthermophilic Archaeon Pyrococcus furiosus**

333 A **tRNA maturation abnormalities connected to stress conditions and transcription deregulation**

722 A **Identification of novel methyltransferases, responsible for N-1 methyl-adenosine base modification of 25S rRNA in *S.cerevisiae***

318-A Mitochondrial poly(A) polymerase is involved in tRNA editing

Mario Fiedler[1], *Elmar Wahle*[1], *Christiane Rammelt*[1]

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Transcription of the mitochondrial genome results in long polycistronic precursors, which are processed mainly by endonucleolytic release of the tRNAs that are interspersed between rRNAs and mRNAs. Interestingly, in most metazoan mitochondrial genomes, genes for some tRNAs overlap with downstream genes encoded on the same strand: in the case of human mitochondria the genes for $tRNA^{Tyr}$ and $tRNA^{Cys}$ share one nucleotide. The processing of the precursor transcript releases a complete $tRNA^{Cys}$ whereas $tRNA^{Tyr}$ lacks the 3' terminal nucleotide, the so-called discriminator [1]. This nucleotide has to be added before the tRNA can be matured by addition of the CCA end and subsequently aminoacylated. The enzyme responsible for this editing reaction has not been identified so far. Since the overlapping nucleotide is an adenosine, we tested whether the mitochondrial poly(A) polymerase (PAPD1) is involved in the reaction. We show that the $tRNA^{Tyr}$ lacking the discriminator is a substrate for PAPD1 *in vitro*. *In vivo*, knock-down of PAPD1 leads not only to a decrease of oligoadenylated tRNA species, but also to a decrease of mature $tRNA^{Tyr}$ carrying both the correct discriminator as well as the CCA end, whereas the number of tRNA species lacking the discriminator and the CCA end was increased correspondingly. Therefore, we suggest that PAPD1 is the enzyme responsible for the addition of the discriminator base.

1. Reichert, A., Rothbauer, U. & Mörl, M. Processing and editing of overlapping tRNAs in human mitochondria. *J. Biol. Chem.* **273,** 31977–31984 (1998).

321-A Structure and Kinetic Mechanism of Protein-only RNase P from A. thaliana

Michael Howard[1], *Markos Koutmos*[2], *Carol Fierke*[1]

[1]University of Michigan; [2]Uniformed Services University of the Health Sciences

Ribonuclease P (RNase P) catalyzes the maturation of the 5' end of tRNA precursors. Typically these enzymes are ribonucleoproteins with a conserved RNA component responsible for catalysis. However, protein-only RNase P (PRORP) enzymes process precursor tRNAs in human mitochondria and in all tRNA-using compartments of *Arabidopsis thaliana*. PRORP enzymes are nuclear encoded and conserved among many eukaryotes. Here we report the crystal structure of PRORP1 from A. thaliana at 1.75 Å resolution, revealing a prototypical metallonuclease domain tethered to a pentatricopeptide repeat (PPR) domain by a structural zinc-binding domain. The metallonuclease domain is a novel high-resolution structure of a Nedd4-BP1, YacP Nucleases (NYN) domain that is a member of the PIN domain-like fold superfamily, including the FLAP nuclease family. In order to understand how this new family of metallonucleases function a kinetic mechanism is required. A minimal kinetic mechanism was derived from measuring steady-state, single-turnover, and binding kinetics. These data suggest a two-step binding mechanism, followed by cleavage of the phosphodiester bond with a maximal rate constant of 4 min^{-1}. Product release is faster than phosphodiester bond hydrolysis and is not the rate-limiting step under steady-state conditions. These studies allow for a molecular-level comparison of the catalytic strategies used by the only known naturally evolved protein and RNA-based catalysts that perform the same biological function, pre-tRNA maturation, thereby providing insight into the differences between the prebiotic RNA world and the present protein-dominated world.

324-A Identification of proteins co-purifying with the yeast RNA exosome and their effect on the complex stabilization

Rogerio Lourenco[1], Fernando Gonzales[1], Carla Oliveira[1]

[1]Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, Brazil

The eukaryotic RNA exosome is a conserved multiprotein complex essential for processing, degradation, and quality control of a wide variety of RNAs. The complex is formed by a nine-subunit core that associates with two hydrolytic 3'-5' exoribonucleases. The involvement of the exosome in different RNA processing pathways suggests that protein cofactors are required for directing the complex to its substrates. We have previously characterized the functions of nuclear proteins that interact with the exosome and modulate its function in the pre-rRNA processing pathway, and are currently interested in identifying other proteins that might associate with the exosome and influence its assembly, stability and activity. Therefore, we have carried out protein co-immunoprecipitation experiments by means of the TAP method, followed by anion-exchange or size-exclusion chromatography. According to mass spectrometry analyses, the exosome core subunits were co-purified with the tagged subunits. These purified complexes are being used in complex stability analyses and RNA degradation assays to evaluate their *in vitro* activity. In addition, we are testing other purification conditions to investigate whether amino acid substitutions in one of the core subunits affect the ability of the exosome to interact with distinct cofactors.

Supported by FAPESP

327-A Absence of a Universal Element for tRNAHis Identity in Eucarya

Bhalchandra Rao[1], *Jane Jackman*[1]

[1]The Ohio State University

Stringent discrimination between tRNA substrates by cognate aminoacyl-tRNA synthetase (aaRS) is essential for high fidelity gene expression. Identity elements within tRNAs aid this discrimination at a kinetic level. The identity element for histidyl tRNAs ($tRNA^{His}$), an extra guanosine at its 5'-end (G_{-1}), is essential for recognition and efficient histidylation by HisRS and its occurrence is conserved across bacteria, archaea and eukarya. In eukaryotes, the G_{-1} is acquired post-transcriptionally by an unusual 3'-5' addition reaction catalyzed by the essential Thg1 enzyme which was first identified in *S. cerevisiae*. Due to the critical nature of the G_{-1} identity element, the presence of a Thg1 gene is widespread in eukaryotes and until recently, no examples of eukaryotic histidyl-tRNAs that lack the essential G_{-1} had been identified.

We recently investigated several eukaryotes, including *Acanthamoeba castellanii* and *Typanosoma brucei* that lack any identifiable Thg1 homolog. Sequencing of $tRNA^{His}$ isolated from these organisms revealed that they lack the universally conserved G_{-1} residue and thus appear to be exceptions to the general rule for $tRNA^{His}$ identity. Due to the previously well-characterized dependence on the G_{-1} residue for recognition by HisRS, we tested the effect of G_{-1} on aminoacylation activity catalyzed by purified *A. castellanii* HisRS (AcHisRS) and *T. brucei* HisRS (TbHisRS) and observed that the presence of G_{-1} is not required for efficient histidylation by AcHisRS and TbHisRS. Yeast genetic complementation and steady state kinetic assays confirm that the recognition of $tRNA^{His}$ by AcaHisRS at the cellular and kinetic level is truly independent of G_{-1} suggesting an alternate mechanism for $tRNA^{His}$ recognition in comparison to the other eukaryotic HisRS. Hence, here we report the first instances of a $tRNA^{His}$/HisRS pair which do not conform to previously accepted dogma for $tRNA^{His}$ identity and thus set the stage for further investigations of the co-evolution of this G_{-1} residue for $tRNA^{His}$ identity, role of Thg1 enzymes in $tRNA^{His}$ maturation and mechanisms for substrate recognition by HisRS.

330-A A Protease that Cleaves the C-terminal Domain of the RtcB-type RNA Ligase in the Hyperthermophilic Archaeon Pyrococcus furiosus

Asako Sato[1], Takeshi Masuda[1], Masaru Tomita[1], Takashi Itoh[2], Akio Kanai[1]

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RNA ligase is an enzyme that can catalyze the ligation of RNA molecules through phosphodiester bond formation. So far, three heat-stable RNA ligases are known in the hyperthermophilic archaeon, *P. furiosus*. The three enzymes are: (1) a putative 2', 5' RNA ligase (PF0027 protein), (2) an RtcB-type RNA ligase (PF1615 protein) and (3) a T4-type RNA ligase (PF0353 protein). The RtcB-type RNA ligase is reported as the missing component in tRNA maturation and the enzyme is able to ligate spliced tRNA halves into mature-sized tRNAs (Englert, M. *et al. PNAS* 2011).

Using purified recombinant RtcB RNA ligase as a substrate, we detected a protease activity in the *Pyrococcus* whole-cell extract that specifically cleaved the C-terminal portion (approximately 5 kDa) of the RtcB RNA ligase. No cleavage of the other two RNA ligases was observed. Protease inhibitors suggested that the responsible enzyme was a chymotrypsin-like serine protease, and gel filtration analysis of the whole cell extract showed that the protease activity was eluted at approximately 400 kDa. Moreover, gel filtration combined with western blotting analysis showed that *Pyrococcus* RtcB ligase formed a protein complex in the cell, and Blue-native PAGE analysis also suggested that the purified *Pyrococcus* RtcB RNA ligase formed a homo-oligomer complex. Since the RtcB RNA ligase lacking the C-terminal portion was able to form the homo-oligomer complex, the C-terminal portion was suggested to be not involved in the oligomerization activity. Meanwhile, the expression of the recombinant RtcB RNA ligase lacking the C-terminal portion became lower in *Escherichia coli* and the purified enzyme lost the RNA ligation activity. Our results suggest that a protease in *Pyrococcus* can cleave the RtcB RNA ligase and regulate its activity.

333-A tRNA maturation abnormalities connected to stress conditions and transcription deregulation

Dominika Wichtowska[1], *Anita K. Hopper*[2], *Magdalena Boguta*[1]

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The normal processing order for transfer RNA (tRNA) in yeast generally starts with cleavage of 5' leader sequence from primary tRNA transcripts; this is then followed by removal of the 3' trailer sequence and, finally, by pre-tRNA splicing. We identified an unanticpated tRNA species which migrated slower than the species commonly assumed to be the primary transcript. This aberrant RNA was observed in wild type strain when the cells were propagated on nonfermentable carbon source or at elevated temperature; the aberrant tRNA was also detected in *rex1*Δ strain which is defective in the 3'-5' exoribonuclease that participates in 3' end processing. Preliminary sequencing indicates that the aberrant tRNA species from wild type cells results from maturation defects at the 3' end rather than from Pol III transcription termination defects. Additional studies show that there is diversification of this processing step among the different tRNA families. We also studied tRNA processing in cells lacking Maf1, the general negative regulator of Pol III transcription. Interestingly, when *maf1*Δ cells were grown on nonfermentable carbon sources, they generated end-processed intron-containing tRNAs species which migrated faster than pre-tRNAs from wild type cells. The migration of the aberrant tRNAs and other intermediates detected from *maf1*Δ cells suggests that there is/are alternative pathway(s) for tRNA maturation or degradation in the absence of Maf1. We are in the process of conducting large scale sequencing of tRNAs isolated from *maf1*Δ cells.

722-A Identification of novel methyltransferases, responsible for N-1 methyl-adenosine base modification of 25S rRNA in *S.cerevisiae*

Sunny Sharma[1, 2], *Christian Peifer* [1, 2], *Peter Kötter* [1, 2] *and Karl-Dieter Entian* [1, 2]

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S.cerevisiae contains two N-1 methyl adenosine (m^1A) base modifications in the helix 25.1 and helix 65 of 25S rRNA. The enzymes responsible for these base modifications remained elusive for a long time. We identified ribosomal RNA processing protein 8 (Rrp8) and Bmt2, a previously uncharacterised ORF as a methyltransferases involved in m^1A645 and m^1A2142 modification of 25S rRNA, respectively. The genes were identified by RP-HPLC screening of all deletion mutants of putative RNA methyltransferase and was confirmed by gene complementation and phenotypic characterization. Furthermore, we analysed the significance of m^1A645 and m^1A2142 modification in ribosome synthesis and translation. Intriguingly, the loss of m^1A645 modification retards the cell growth at lower temperature and cause significant defect in 60S assembly. On the other hand loss of m^1A2142 modification confers anisomycin and hydrogen peroxide hypersensitivity to the cells. Our results emphasize the importance of RNA modifications in cellular physiology.

Ribosomes and Translation

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 336 A – 360 A
Location: Main Hallway & Sanada Foyer

336-A Characterization of Rbtf1 and its role in ribosome synthesis

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Ribosome biogenesis is essential for the translational capacity of a cell and its regulation is tightly linked to the rate of cellular growth. Synthesis of ribosomes is one of the most energy consuming cellular processes, as these macromolecular machineries are present in high abundance and built up of a complex structure of RNA and proteins. Ribosome biogenesis includes transcription of rRNA, expression of ribosomal proteins and the activity of ribosomal maturation factors, which are processing rRNA and assembling ribosomal particles. In human cells, the regulatory network upstream of ribosome synthesis appears very complex; yet little is known as to how the various nutrient and growth factor induced signaling pathways cooperate to regulate ribosome synthesis.

We have recently performed a genome-wide siRNA screen to identify the cellular repertoire of factors involved in human 40S subunit biogenesis. The screen identified a number of candidate proteins that control ribosome synthesis, including the uncharacterized, putative transcription factor Rbtf1. We show that Rbtf1 is essential for both 40S and 60S subunit production. Rbtf1 is a nucleoplasmic protein, which is not required for rRNA transcription by RNA polymerase I, but for nuclear maturation of ribosomal subunits. Based on these data, we are testing how Rbtf1 influences ribosome synthesis by its potential function as a transcription factor and how Rbtf1 could link upstream signaling pathways to ribosome biogenesis.

339-A Escherichia coli ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation

Mélodie Duval[1], Alexey Korepanov[2], Olivier Fuchsbauer[1], Pierre Fechter[1], Ronald Micura[4], Bruno Klaholz[3], Stefano Marzi[1], Pascale Romby[1]

[1]IBMC-CNRS, Strasbourg; [2]IBPC-CNRS, Paris; [3]IGBMC, Illkirch; [4]Leopold Franzens University, Innsbruck

Many bacterial mRNAs adopt structures in their 5' untranslated regions that modulate the accessibility of the 30S ribosomal subunit. Structured mRNAs interact with the 30S in a two-step pathway where the docking of a folded mRNA precedes an accommodation step. Here, we demonstrate that ribosomal protein S1 endows the 30S with an RNA chaperone activity that is essential for the docking, the unfolding of structured mRNAs, and the correct positioning of the initiation codon inside the decoding channel. The first three OB-fold domains of S1 retain all the activities of the protein on the 30S subunit. We show that S1 is not required for all mRNAs and acts differently on mRNAs according to the signals present at their 5' ends. Therefore, S1 confers activities to the ribosome that are adapted for a given mRNA creating heterogenous populations of ribosomal initiation complexes to selectively translate unstructured and structured mRNAs.

342-A Cryo-EM structure of the mammalian ribosomal 43S preinitiation complex

Yaser Hashem[1], Amedee Des Georges[2], Vidya Dhote[3], Robert Langlois[1], Robert Grassucci[1], Christopher U.T. Hellen[3], Tatyana V. Pestova[3], Joachim Frank[2]

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Protein translation initiation in eukaryotes is a very complex process that involves a dozen of initiation factors and begins by the assembly of the 43S preinitiation complex. The process starts by the formation of the ternary complex (TC) from Met-tRNA$_i^{Met}$, eukaryotic initiation factor 2 (eIF2) and GTP. Then the TC along with eukaryotic initiation factors 3, 1 and 1A (eIF3, eIF1 and eIF1A respectively) cooperatively bind to the small ribosomal subunit subunit yielding the 43S preinitiation complex that is ready to bind the messenger RNA (mRNA) and scan for the initiation codon. Scanning on structured mRNAs in higher eukaryotes requires in addition the presence of DHX29, a DExH-box protein that also binds directly to the 40S subunit. The assembly of most of these factors on the 40S subunit, and the structure of the 43S complex more generally, remained so far elusive to structural studies and has been subject to numerous speculations. Here, we present the first cryo-electron microscopy structure of the mammalian DHX29-bound 43S complex (figure1 below). It reveals the assembly of the 43S complex and the binding sites of its different initiation factors including eIF3. Our structure reveals that eIF2 interacts with the 40S subunit via its alpha subunit and supports Met-tRNA$_i^{Met}$ in a novel eukaryotic P/I orientation (eP/I). The structural core of eIF3 resides on the back of the 40S subunit establishing a rather reduced interaction surface with the latter through two principal points of contact only, whereas DHX29 binds around helix 16. Although the assignment of eIF3 subunits is still controversial, our structure localizes at least three peripheral subunit of eIF3, thus representing the most complete structure of eIF3 to date. In addition, we discuss eIF3 binding on the 40S subunit in the context of different relevant complexes such as 40S-HCV IRES complex and the small ribosomal subunit from ribosomes of kinetoplatids, presenting extraordinarily large expansion segments. In conclusion, our structure provides insights into eukaryote-specific aspects of translation, including the mechanism of action of different initiation factors such as DHX29.

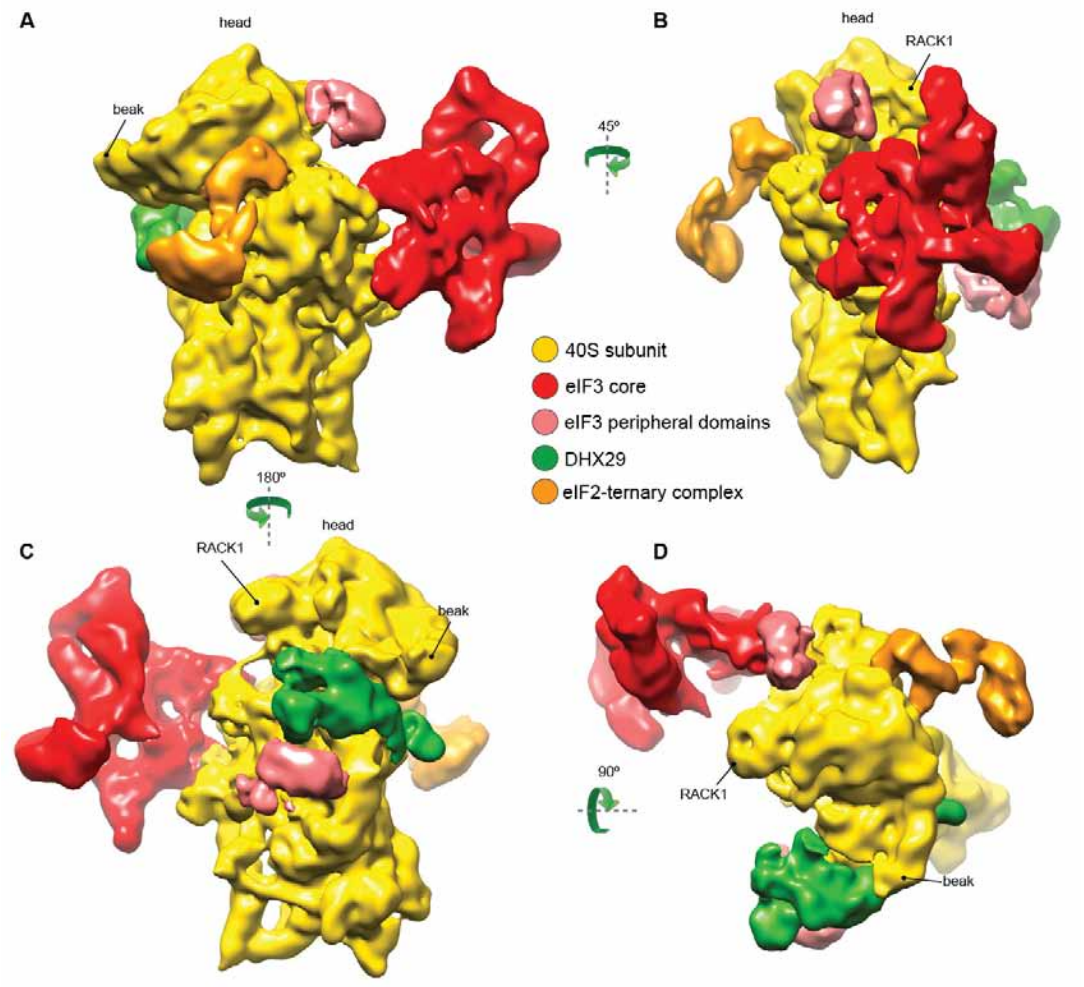
References:

Hashem et al. Structural insights into the mammalian ribosomal 43S preinitiation complex**.** *Cell* 2013 (in press)

Hashem et al. High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome. *Nature* 2013

Siridechadilok et al. Structural Roles for Human Translation Factor eIF3 in Initiation of Protein Synthesis. *Science* 2005

Spahn et al. Hepatitis C Virus IRES RNA-Induced Changes in the Conformation of the 40S Ribosomal Subunit. *Science* 2001



345-A The intimate connection of RNA granules with human 4Es changes with use of different protein variants

Klára Kazdová[1], Katerina Mocová[1], Tomáš Mašek[1], Silvia Mrvová[1], Martin Pospíšek[1]

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Three different eukaryotic translation initiation factors belonging to the 4E family are present in human cells. First, eIF4E1, which directs ribosome to the mRNA cap structure by recognizing it via residues Trp56 and Trp102. 4E1 initiates translation via interaction with the scaffolding protein eIF4G and is tightly controlled by 4E-binding proteins 4E-BP1,2,3. Second, eIF4E2, which possesses characteristic substitutions of Trp56 and Trp43 for tyrosines that cause weaker cap binding. In contrast to 4E1, 4E2 does not bind the scaffolding protein 4G, however, it is still able to bind 4E-BPs. Therefore, it is assumably unable to initiate translation and a regulatory role has been assigned to it instead. It establishes anterior-posterior body axis in *Drosophila* embryo by inhibiting caudal and hunchback mRNAs translation and drives embryonic patterning by inhibiting HoxB4 and HoxB8 mRNAs in mouse oocytes. Recently, 4E2 has been shown to play important role in a non-canonical translation initiation during cellular response to hypoxia. Last, 4E3 has the Trp 43 and 56 substituted for Cys or Tyr, which weakens its cap binding even more than that of 4E2. Contrary to 4E2, 4E3 is able to bind 4G but not 4E-BPs and its direct role in 5' mRNA cap recognition in translation initiation thus cannot be excluded. Each of the 4E1,2,3 proteins is present in human cells as several predicted variants arising from alternative mRNA splicing and alterations in transcription starts. We confirmed existence of some of them by cloning their cDNAs from human leukemic cells. To investigate subcellular distributions of the 4E1,2,3 protein variants corresponding to cloned cDNAs, we produced them as N-terminal GFP fusions in the human osteosarcoma cell line (U2OS). To our knowledge, subcellular localizations of only 4E1 and mouse 4E2 have been published till now. Contrary to strictly cytoplasmic distribution of mouse 4E2, we observed nuclear-cytoplasmic distribution of all the human 4E1,2,3 variants tested. Localizations of all the proteins were verified using antibodies against endogenous proteins. We were further interested whether all of the 4E1,2,3 variants form a part of RNA granules and thus could be involved in more general processes associated with mRNA turnover during normal conditions as well as under stresses. We found that during heat shock and oxidative stress both of the 4E1 variants tested localized to P-bodies (PB) and stress granules (SG). Contrary to that, all of the three 4E2 variants localized to PB only and 4E3 was not detected in any of those structures. Moreover, careful quantitative analysis of these data suggested different abilities to associate with PB between different protein variants of both 4E1 and 4E2 translation initiation factors. Our results thus indicate importance of alternative splicing for functional diversity of translation initiation factors belonging to the 4E family.

348-A Function of Fap7 in the maturation of the ribosome small subunit

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Over 200 pre-ribosomal factors are involved in the maturation of ribosomes. Most of these factors are essential to cell survival, but their precise function remains elusive. One of the last steps of maturation of the ribosome small subunit is the cleavage of 20S pre-rRNA in 18S rRNA in the cytoplasm. This cleavage is carried out by the endonuclease Nob1 and also requires the presence of other factors such as the methyltransferase Dim1, and a plethora of NTPases including the Rio protein kinases, Prp43 and its cofactor Pfa1, the Ltv1 GTPase and the Fap7 NTPase.

The function of Fap7 is especially intriguing since the human homologue bears adenylate activity, an enzymatic activity not usually found during ribonucleoprotein biogenesis. In addition, the function of Fap7 is intimately linked to its interaction with the Rps14 ribosomal protein. The Rps14 C-terminal domain is essential for D-site cleavage and is located in proximity to the 18S rRNA 3'-extremity in the mature ribosome. The deletion of this protein causes the 5q syndrome that is phenotypically close to Diamond Blackfan anemia. The link between the enzymatic activity of Fap7 and its role in ribosome biogenesis remains enigmatic.

We have conducted functional and structural characterisation of the Fap7 protein alone and in complex with Rps14 and nucleotides. Using a combination of structural studies by X-ray crystallography, small angle X-ray scattering (SAXS) in solution, enzymatic studies on purified proteins, and *in vitro* D site cleavage reaction assays on purified pre-ribosomes, we were able to uncover the function of Fap7 within pre-40S ribosomes. We show that the Fap7/Rps14 interaction is involved in a major conformational change at the heart of the pre-ribosomes and that this structural rearrangement is necessary to expose the D-site for cleavage by the endonuclease Nob1. The link between the enzymatic activity and the conformational switch both before and after cleavage is currently been investigated.

351-A The communication between ribosome biogenesis and cell cycle machinery

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Ribosome, the universal machine for decoding the genetic code into polypeptides, is a complex of RNAs and proteins that comprises over 50% of the cell mass in rapidly growing cells. Thus ribosome biosynthesis is required to support the growth and proliferation of cells and consumes a huge amount of cellular energy and other resources. Disruption of normal ribosome biogenesis affects cellular growth and can result in cell abnormalities in proliferative cells. In humans these abnormalities are classified as 'ribosomopathies' and includes Diamond Black Fan Anemia, Shwachman Diamond syndrome, and increased propensity for some cancers. Our lab is using budding yeast to establish basic parameters for the connection between disruption of ribosome biosynthesis and cell cycle progression. So far, we have studied cell cycle progression, cell morphology and bud site selection after the repression of the synthesis of 54 of the 79 ribosomal proteins.

Interestingly, repression of the synthesis of specific ribosomal proteins generates different responses, including arrest (or delay) in G1 or G2/M stage of the cell cycle. Cell morphology includes cells with elongated buds, cells with more than one bud, and increased size of mother cells. Furthermore, repression of synthesis of new ribosomal proteins destabilized the existing pool of ribosomes. Since responses specific to the repression of individual ribosomal protein genes results in a variety of phenotypes, these effects do not originate from just reduced protein synthesis capacity. We are currently investigating possible explanations, including abnormal localization and stability of the yeast cell cycle-dependent kinase Cdc28 and other cell cycle proteins and their mRNAs.

354-A Sequence-specific targeting of bacterial ribosomal RNA as a way to look for inhibition pockets

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Many antibiotics inhibit protein synthesis in bacteria by interacting with their ribosomal RNA [1]. Unfortunately, bacteria have developed various resistance mechanisms to fight back existing drugs. Also, in most cases, the known antibiotics lack specificity toward pathogenic bacteria. Designing new scaffolds or modifying the known compounds has been of moderate success and most importantly will not eliminate the cross-resistance of related antibiotics. To design effective inhibitors of bacterial translation one has to explore new sites and possibly unknown inhibition mechanisms.

We have been looking for good inhibition sites by rational screening of the ribosomal RNA with short modified oligonucleotides as probes. The aim is to evaluate the effectiveness of these antisense single-stranded oligonucleotides in inhibiting translation by binding to ribosomal RNA in a sequence-dependent manner. The design of oligonucleotide sequences is based on the atomic-resolution structures of the ribosomes [2], bioinformatics analyses, and molecular dynamics simulations. Not only sequence complementarity but also the secondary and tertiary structure, flexibility and other physicochemical descriptors of ribosomal RNA and modified oligonucleotides are taken into account in the design process.

Next, we check the ability of such oligomers to inhibit protein synthesis *in vitro* in an optimized by us *E. coli* cell-free transcription/translation system with ß-galactosidase as a reporter protein. The oligonucleotides that have been tested are 2'O-methyl-RNA, peptide nucleic acid and locked nucleic acid. For example, we have identified potential 2'O-methyl-RNA oligonucleotides (10-15 nucleotides long) that block the function of certain fragments of ribosomal inter-subunit bridges through steric hindrance, thereby halting protein synthesis. If the oligomer target site is in proximity to known antibiotic binding site we also check the synergistic (or antagonistic) effect of their combinations. To further investigate the mode of binding and confirm strand invasion of oligonucleotides we perform fluorescence spectroscopy, isothermal titration calorimetry and thermal denaturation studies on selected isolated ribosomal RNA fragments.

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References:

1. Wilson, D. N. The A–Z of bacterial translation inhibitors. Crit. Rev. Biochem. Mol. Biol., 44, 393–433, 2009

2. Wimberly, B. T. The use of ribosomal crystal structures in antibiotic drug design. Curr. Opin. Investig. Drugs, 10, 750–765, 2009

357-A A NEW ROLE FOR NOT5 OF THE CCR4-NOT COMPLEX IN CONNECTING TRANSCRIPTION WITH TRANSLATION

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The Ccr4-Not complex contributes to both transcription and cytoplasmic mRNA decay and is found both in the cytoplasm and in the nucleus[1], like the Rpb4 subunit of RNA polymerase II, recently reported to play a role in translation by interacting with the translation scaffold factor eiF3[2]. In our work we show that Not5 is involved in the crosstalk between transcription and translation by contributing to the nuclear-cytoplasmic shuttling of Rpb4 and to the assembly of RNAPII on translating ribosomes. Indeed, first we found that the transctipion factor Rpb4 and translation factor eiF3 interactions are dependent upon the Not5 subunit of the Ccr4-Not complex. This can be explained by the fact that Not5 is required for the presence of Rpb4 in translating polysomes and in fact for the appropriate accumulation of Rpb4 in the cytoplasm under relevant conditions altogether. Second, besides Rpb4, several other polymerase subunits (Rpb1, Rpb2, Rpb9, Rpb11) are present in translating ribosome fractions, compatible with the publised data showing that assembly of the polymerase is cytoplasmic and with the new idea that this is co-translational. Not5 is not only required for Rpb4 cytoplasmic accumulation, but also for appropriate co-translational interaction of newly synthesiezd Rpb1 with its chaperones. Consequently RNA polymerase II is not appropriately assembled in cells lacking Not5, and subcomplexes containing various polymerase subunits accumulate. Hence Not5 of the Ccr4-Not complex is central to the integration of transcription and translation into a system that efficiently controls eukaryotic gene expression.

References:

1. Collart MA, Panasenko OO. Gene 15: 42-53 (2012)
2. Harel-Sharvit et al., Cell 143: 552-563 (2010)

360-A Transcription regulation by cAMP-CRP of the rmf gene for 100S ribosome formation

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In proteobacteria group gamma including *Escherichia coli*, protein synthesis is suppressed by the formation of 100S ribosomes under stress conditions such as nutrient starvation. The 100S ribosome, a dimer of 70S ribosomes, is formed after association of 70S ribosome monomer with "Ribosome Modulation Factor" (RMF) [1]. Upon entry into the stationary growth phase, the 70S ribosomes are converted into 100S dimers, which are functionally inactive in translation. An *E.coli* strain deficient in the *rmf* gene cannot form 100S ribosomes and its lifetime is shorter than that of the wild-type strain, indicating that the transformation of ribosomes is an important strategy for *E.coli* survival under stress conditions.This ribosomal resting state is called the hibernation stage [2]. At present, however, little is known regarding the regulation of stationary-phase-coupled RMF expression.

After Genomic SELEX screening *in vitro* of regulation targets of CRP (cAMP receptor protein), the global regulator of genes for carbon source utilization, the *rmf* gene was predicted to be under the direct control of cAMP-CRP [3]. In order to confirm the regulation *in vivo* of *rmf* by cAMP-CRP, we investigated the effects of cAMP and CRP for the expression of RMF and the formation of 100S ribosome by using the deletion mutants of *cyaA* and *crp* genes. The results altogether, indicated the decreases in RMF production and 100S ribosome formation in the absence of cAMP-CRP. We concluded that cAMP-CRP is involved in transcription regulation of the *rmf* gene and the formation of 100S ribosome [4]. In addition, we will discuss other factors involved in ribosome dimerization.

[1] Kato T, Yoshida H, Miyata T, Maki Y, Wada A, Namba K. 2010. Structure of the 100S Ribosome in the Hibernation Stage Revealed by Electron Cryomicroscopy. *Strucuture*. **18**:719–724.

[2] Yoshida H, Maki Y, Kato H, Fujisawa H, Izutsu K, Wada C, Wada A. 2002. The ribosome modulation factor (RMF) binding site on the 100S ribosome of *Escherichia coli*. *J. Biochem*. **132**:983–989.

[3] Shimada T, Fujita N, Yamamoto K, Ishihama A. 2011. Novel roles of cAMP receptor protein (CRP) in regulation of transport and metabolism of carbon sources. *PLoS ONE.* **6**:e20081.

[4] Shimada T, Yoshida H, Ishihama A. 2013. Involvement of cyclic AMP receptor protein in regulation of the *rmf* gene encoding the ribosome modulation factor in *Escherichia coli*. *J. Bacteriol.* **195**:in press.

Translational Regulation

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 363 A – 399 A
Location: Main Hallway & Sanada Foyer

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363 A **A tale of two termini: profiling mRNA 5'-3' interactions in vivo.**
366 A **Study of PTBP1-RRM12 in complex with an RNA-stemloop of the EMCV-IRES**
369 A **Inactivation of the mTORC1-eIF4E Pathway alters Stress Granules Formation**
372 A **Changes in polysomal profiles in cells with aberrant RNA degradation**
375 A **Observation of mRNA Surveillance in Living Yeast by Ribosome Profiling**
378 A **Activation of HRI Kinase and Translation Control by Oxidative Stress**
381 A **Posttranscriptional control of the DNA damage response through TIAR**
384 A **Global translational control during norovirus infection**
387 A **Caspase-3 cleaves hnRNP K in erythroid differentiation**
390 A **Translation of human LAT2 mRNA is controlled by a short upstream open reading frame**
393 A **Regulation of translation of the most abudant protein in human body, type I collagen.**
396 A **The role of human RLI in cell proliferation and translational regulation**
399 A **Tdrd7, a RNA binding protein, acts to restrict Epha2 protein synthesis in space and time during lens development**

363-A A tale of two termini: profiling mRNA 5'-3' interactions in vivo.

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Interactions between the 5' and 3' termini of mRNAs have long been postulated to occur in the cell. This "closed-loop" model of mRNA is attractive from a number of viewpoints: functionally, the closed loop explains the ability of 3' UTR features to modulate events occurring at the 5' end such as translation initiation, while mechanistically, the 5' cap structure is known to bind the eIF4F complex, which also interacts with the poly(A)-tail via PABP, conceptually forming a closed loop mRNP if all interactions occur simultaneously.

Here we introduce a novel assay to detect the closed-loop conformation of specific mRNAs *in vivo* for the first time. Using *Saccharomyces cerevisiae*, we demonstrate that the closed-loop is the predominant conformation found in mRNAs bound by eIF4F, but also introduce the possibility that this structure is not adopted by all mRNAs during normal growth. While the closed-loop conformation has been reconstituted *in vitro* by mixing eIF4F and PABP with mRNA, the situation may be different in the cell. Various *in vivo* examples are known where interactions between the eIF4F subunits and PABP come under regulatory intervention, either globally or in a transcript-specific manner, resulting in reduced translational efficiency. Thus, detecting altered closed-loop status represents a catch-all method of identifying whether mRNA is under translational regulation. We are investigating the global and transcript-specific regulation of the closed-loop conformation in response to environmental cues, which will identify regulatory mechanisms that have been expanded upon during the evolution of more complex organisms to rapidly fine-tune gene expression.

366-A Study of PTBP1-RRM12 in complex with an RNA-stemloop of the EMCV-IRES

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Internal ribosomal entry sites (IRESes) are large RNA structures present in the 5' untranslated region (5' UTR) of many viral and some cellular mRNAs(1). Unlike canonical translation initiation, IRES mediated translation initiation is 5' cap independent but often requires cellular IRES trans-acting factors (ITAFs). The polypyrimidine tract binding protein 1 (PTBP1) is among the most frequently found ITAFs and might modulate the IRES activity by stabilization of the appropriate IRES structure(2).

PTBP1 harbors four RNA recognition motifs (RRMs) of which the 2 N-terminal RRMs, RRM1 and RRM2, act independently whereas the 2 C-terminal RRMs, RRM3 and RRM4, interact with each other, thereby orienting their RNA-binding surfaces in opposite directions(3). Thus, PTBP1 might be able to reorganize the structure of an RNA target by bringing distant binding sites into proximity.

PTB binding sites were mapped on the IRES of encephalomyocarditis virus (EMCV) by hydroxyl radical probing(4). It was found that two molecules of PTBP1 bind to the IRES of EMCV in a unique orientation: One PTBP1-molecule binds the IRES-domains H-L and the second one to domains D-F. The footprinting data suggests that RRM1 binds the UCUUU-pentaloop of the IRES-domain F and RRM2 binds its regular stem. We investigated the binding of RRM12 to domain F by NMR titrations: In contrast, our NMR-data suggest that RRM12 binds the isolated domain F with RRM2 contacting the loop. We present a preliminary structure of RRM2 bound to domain F.

1. Spriggs KA, Stoneley M, Bushell M, Willis AE. 2008. *Biology of the cell / under the auspices of the European Cell Biology Organization* 100: 27-38

2. Song Y, Tzima E, Ochs K, Bassili G, Trusheim H, et al. 2005. *RNA* 11: 1809-24

3. Vitali F, Henning A, Oberstrass FC, Hargous Y, Auweter SD, et al. 2006. *EMBO J* 25: 150-62

4. Kafasla P, Morgner N, Poyry TA, Curry S, Robinson CV, Jackson RJ. 2009. *Mol Cell* 34: 556-68

369-A Inactivation of the mTORC1-eIF4E Pathway alters Stress Granules Formation

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Stress granules (SG) are cytoplasmic multimeric RNA bodies that form under stress conditions known to inhibit cap-dependent translation. SG contain translation initiation factors, RNA binding proteins like the Fragile Mental Retardation Protein (FMRP) and signaling molecules. SG are known to inhibit apoptotic pathways, thus contributing to chemo- and radio-resistance in tumor cells. However, whether stress granules formation involves oncogenic signaling pathways is currently unknown. Herein, we report a novel role of the mTORC1-eIF4E pathway, a key regulator of cap-dependent translation initiation of oncogenic factors, in SG formation. mTORC1 specifically drives the eIF4E-mediated formation of SG through the phosphorylation of 4E-BP1, a key factor known to inhibit formation of the mTORC1-dependent eIF4E-eIF4GI interactions. Disrupting formation of SG by inactivation of mTOR with its specific inhibitor pp242 or by depletion of eIF4E or eIF4GI blocks the SG-associated antiapoptotic p21 pathway. Finally, pp242 sensitizes cancer cells to death *in vitro* and inhibits the growth of chemoresistant tumors *in vivo*. This work therefore highlights a novel role of the oncogenic mTORC1-eIF4E pathway, namely the promotion of formation of antiapoptotic SG.

372-A Changes in polysomal profiles in cells with aberrant RNA degradation

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Non-protein-coding RNAs (ncRNAs) are important in regulation of gene expression in many different ways. Very often they are connected with development, stress response and pathogenesis. One of the less known functions of ncRNA (excluding rRNAs, tRNAs and miRNAs) is the modulation of translation. Long non-coding RNAs for instance have been linked to both repression and stimulation of translation. We are interested in novel tRNA-derived small RNAs (tRFs) that are produced by endonucleases such as DICER, RNaseZ and angiogenin (1, 2). Angiogenin produced tRFs have a role in translational silencing and viral infection (3, 4).

Here we show, that 3' to 5' exoribonuclease DIS3L2 is involved in tRFs processing. DIS3L2 is a sequence homolog of the main exosomal ribonucleases DIS3 and DIS3L, that has been linked to Perlman syndrome (5). DIS3L2 is localized to cytoplasm where it acts on tRFs in exosome-independent manner. Our results indicate that DIS3L2 together with tRFs associates with ribosomes. Overexpression of wild type DIS3L2 in HEK293T cells causes changes in polysome to monosome ratios. We are currently investigating whether DIS3L2 is directly involved in translational regulation.

(1) Haussecker et al. RNA. 2010
(2) Sobala et al. Wiley Interd Reviews RNA. 2011
(3) Ivanov et al. Mol Cell. 2011
(4) Wang et. al. Mol Ther. 2013
(5) Astuti et al. Nat Genet. 2012

375-A Observation of mRNA Surveillance in Living Yeast by Ribosome Profiling

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The synthesis of proteins is a major step in gene expression and therefore represents a regulatory point for modulating cellular output. During elongation, ribosomes are thought to pause along the mRNA transcript upon colliding with various obstacles or translating specific sequence motifs. In addition, ribosomes halt if they arrive at the 3' end of a transcript after failing to properly terminate or if the message is endonucleolytically cleaved. Genetic studies have suggested that the protein Dom34 and a GTPase binding partner, Hbs1, target mRNAs associated with paused ribosomes for degradation (Doma and Parker 2006, *Nature* **440**:461-4). While biochemical data support this idea by showing that Dom34 directly binds and dissociates ribosomes (Shoemaker *et al*. 2010, *Science* **330**:369-72), the natural cellular targets of Dom34 remain unknown. To identify functions of Dom34 *in vivo*, knockout and wild type strains of *S. cerevisiae* were subjected to a high-throughput footprinting analysis (ribosome profiling) that reveals the places where ribosomes are bound throughout the transcriptome. Sites where ribosomes are selectively enriched in the knockout strain relative to those in the wild type strain likely represent places where Dom34 dissociates paused ribosomes. These data reveal that ribosome occupancy on known pauses is not generally enhanced in the strain lacking Dom34. Instead, the targets of Dom34 include a small number of specialized targets in open reading frames. In addition, ribosomes are enriched at the 3' end of many transcripts in the Dom34 deletion strain and generally covere <10 bp of polyadenine at the 3' end of the protected fragment. These data imply that Dom34 dissociates ribosomes that bypass the stop codon and thus serves as a quality control factor required for recycling ribosomes that fail to terminate by the conventional pathway (mediated by eRF1, eRF3, and Rli1). These results support a general role for Dom34 in ribosome recycling in addition to a more specialized role on particular gene products.

378-A Activation of HRI Kinase and Translation Control by Oxidative Stress

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Oxidative stress causes rapid inhibition of cellular protein synthesis through phosphorylation of the eukaryotic translation initiation factor 2a (eIF2a) by the heme-regulated inhibitor (HRI) kinase. Arsenite is a potent inducer of oxidative stress, and arsenite-induced translation inhibition leads to the assembly of stalled mRNAs in cytoplasmic stress granules (SGs). In line with the central role of HRI as a mediator of translation suppression, we observe that cells lacking HRI do not form SGs after arsenite treatment. The mechanism by which HRI is activated under conditions of oxidative stress, however, is not understood.

To address the mechanism of HRI activation, we asked whether HRI might undergo thiol conjugation under oxidative conditions. To this end, HEK293 cells were transfected with YFP-HRI and subjected to control conditions or oxidative stress. Prior to lysis, cells were treated with N-ethylmaleimide (NEM), which binds free thiols and thus prevents disulfide bond formation post lysis. YFP-HRI was immunoprecipitated and analyzed by western blot under non-reducing conditions, whereby disulfide bonds formed inside cells are retained. Our results show that HRI forms a covalent, high molecular weight complex, most likely a dimer, in cells exposed to arsenite- and hydrogen peroxide-induced oxidative stress. Further evidence for HRI dimerization was obtained by co-immunoprecipitation of HA-tagged HRI with YFP-HRI. Mass spectrometry analysis showed that HRI kinase is the only major component of the high molecular weight complex, compatible with HRI dimerization. Interestingly, we found that HRI forms a non-covalent dimer under normal conditions, whereas the covalent complex occurs only during oxidative stress, most likely by disulfide bond formation. Our results suggests that dimerization of HRI may be important for kinase activation. We are currently in the process of identifying cysteine residues involved in covalent complex formation, which will help us to fully understand the mechanism by which HRI is activated under conditions of oxidative stress.

381-A Posttranscriptional control of the DNA damage response through TIAR

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The RNA-binding proteins TIA1 and TIAR regulate splicing of pre-mRNAs in the nucleus and repress translation of mRNAs in the cytoplasm. In response of UVB treatment, cells reduce the global rate of protein synthesis, and TIAR was shown to mediate inhibition of translation after UVB. While UV induces single-strand DNA breaks that are repaired through nucleotide excision repair, g-radiation causes more severe double-strand breaks, which require homologous recombination or error-prone non-homologous end-joining for repair.

To date, regulation of translation in response to G-radiation has not been investigated. Using human HCT116 colon carcinoma cells, we found that the global rate of translation is attenuated in response to low dose G-radiation in a TIAR-dependent manner. Importantly, knock down of TIAR reduces activation of the checkpoint kinase Chk2 and specifically impairs maintenance of the G2/M checkpoint in response to G-radiation. In the absence of TIAR, cells exposed to G-radiation enter M-phase prematurely and show enhanced proliferation rates. This goes along with strongly increased numbers of micronuclei, which result from the failure to properly segregate damaged chromosomes. When we combined TIAR depletion with p53 deletion, we observed an additive effect on checkpoint de-regulation, indicating that TIAR is an important component of a p53-independent checkpoint mechanism. We have identified putative targets of TIAR involved in G2/M checkpoint maintenance by deep sequencing of TIAR-associated mRNAs. This approach is combined with the analysis of mRNAs whose translation is specifically altered in the absence of TIAR. Taken together, out data provide evidence for the important role of a posttranscriptional, TIAR-dependent mechanism in the maintenance of genome integrity.

384-A Global translational control during norovirus infection

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Human norovirus (HuNV) is a member of the calicivirus family and is responsible for more than half of the viral gastroenteritis outbreaks, infecting over one million people last winter in Great Britain. Due to the lack of a suitable cell culture system, its replication mechanisms are poorly understood but two animal caliciviruses, the feline calicivirus (FCV) and mouse norovirus (MNV) provide models to increase our understanding of norovirus translation and replication.

Caliciviruses possess a positive-sense, single-stranded RNA genome of 7-8 kb, which functions as mRNA. Unlike most cellular mRNAs, the calicivirus RNA genome does not possess a 5' cap structure but instead has a 13–15 kDa viral protein, genome linked (VPg) acting as a novel proteinaceous "cap substitute" to direct translation and hijack the host protein synthesis machinery. In addition, viruses can silence the expression of genes involved in the anti-viral responses and the viral life cycle completion requires separated events occurring at different times since viral transcripts are used as template for both translation (mRNA) or replication (genomic RNA). Recent evidence suggests that the dynamic nature of both host and viral mRNA expression is a key coordinator of viral pathogenesis, with different host genes expressed at different times during infection, regulated through their storage and/or decay in subcellular compartments such as stress granules, to stall their translation, or processing bodies (P-bodies), for their further degradation. Viral proteins can also be found in these compartments, suggesting an important interplay between RNA turnover and viral life cycle.

Therefore to investigate global translational control during norovirus infection, and dissect host-pathogen interactions, we adopted a three-pronged strategy:

1. We analysed the regulation of translational factors during MNV and FCV infection and observed changes in the phosphorylation levels of eIF4E, eIF2alpha and 4E-BP1, and identified how MAPK and mTOR signalling pathways are controlling these events.

2. We investigated how infection affects the formation of P-bodies and stress granules and have evidence that MNV and FCV infection modulates the formation of stress granules.

3. We set out to dissect the global regulation of translational control by performing polysome profiling analysis to identify how host mRNAs expression is affected during MNV infection and will provide preliminary results.

The dissection of host-virus interactions during norovirus infection should ultimately contribute to the development and design of new antiviral therapy for this important human pathogen.

387-A Caspase-3 cleaves hnRNP K in erythroid differentiation

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Post-transcriptional control of gene expression is crucial for the control of cellular differentiation. Erythroid precursor cells loose their organelles in a timely controlled manner during terminal maturation to functional erythrocytes. Extrusion of the nucleus precedes the release of young reticulocytes into the blood stream. The degradation of mitochondria is initiated by reticulocyte 15-lipoxygenase (r15-LOX) in mature reticulocytes. At that terminal stage the release of r15-LOX mRNA from its translational silenced state induces the synthesis of r15-LOX. Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a key regulator of r15-LOX mRNA translation. HnRNP K that binds to the differentiation control element (DICE) in the 3' untranslated region (UTR) inhibits r15-LOX mRNA translation initiation. During erythroid cell maturation, activation of r15-LOX mRNA translation is mediated by post-translational modifications of hnRNP K and a decrease of the hnRNP K level. To further elucidate its function in the post-transcriptional control of gene expression we investigated hnRNP K degradation employing an inducible erythroid cell system that recapitulates both nuclear extrusion and the timely controlled degradation of mitochondria mediated by activation of r15-LOX synthesis. Interestingly, we detected a specific N-terminal cleavage intermediate of hnRNP K lacking DICE binding activity that appeared during erythroid differentiation and puromycin induced apoptosis. Employing mass spectrometry and enzymatic analyses we identified Caspase-3 as the enzyme that cleaves hnRNP K specifically. *In vitro* studies revealed that cleavage by Caspase-3 at amino acids (aa) D334-G335 removes the C-terminal hnRNP K homology (KH) domain 3 that confers binding of hnRNP K to the DICE. Our data suggest that the processing of hnRNP K by Caspase-3 provides a save-lock mechanism for its timely release from the r15-LOX mRNA silencing complex and activation of r15-LOX mRNA synthesis in erythroid cell differentiation.

390-A Translation of human LAT2 mRNA is controlled by a short upstream open reading frame

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Ribosome profiling has shown that ~25% of mammalian mRNA transcripts contain ribosome densities on short upstream open reading frames (uORFs) in the 5' UTR. The functions and mechanisms of these uORFs are still poorly defined. Human LAT2 hydrophobic amino acid transporter mRNA is an example of a uORF containing transcript. A short polypeptide composed of ~40% phenylalanine and leucine is encoded by the LAT2 uORF.

We are investigating how this uORF regulates the translation of the LAT2 gene. Mutation of the uORF AUG start codon to AUC or AAG significantly decreases translation of the LAT2 protein coding open reading frame *in vivo*. Toeprinting experiments demonstrate uORF translation *in vitro*. These results are in contrast to known models of uORF function wherein uORF translation is generally inhibitory to translation and elimination of uORF would lead to increased levels of translation. To elucidate the mechanism of uORF dependent translational regulation of LAT2, we performed additional toeprinting and photo-crosslinking experiments which indicate that the LAT2 5' UTR can recruit ribosome independently of a 5' cap. An additional ribosome binding site seems to be present between the uORF and the start of the LAT2 coding sequence. We propose a model where an inactive ribosome is initially bound within the LAT2 5'UTR. 5' Cap dependent translation of the uORF by a second ribosome leads to RNA structural switch and thereby activates the first ribosome. We are working on the validations of this model *in vitro* and *in vivo*.

393-A Regulation of translation of the most abudant protein in human body, type I collagen.

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Type I collagen is the most abundant protein in human body and is composed of two a1(I) and one a2(I) polypeptides. During wound healing or in fibrosis the synthesis of type I collagen is increased several hundred fold, predominantly due to increased half life and more efficient translation of collagen mRNAs. A unique stem-loop structure in the 5'UTR of collagen mRNAs (5'SL) regulates translation by binding LARP6. LARP6 recruits positive and negative regulators of translation to collagen mRNAs to balance the synthesis of a1(I) and a2(I) chains. RNA helicase A (RHA) is recruited to facilitate translation initiation, without RHA collagen mRNAs can not be loaded onto polysomes. LARP6 also associates collagen mRNAs with vimentin and nonmuscle myosin filaments. Binding to vimentin stabilizes collagen mRNAs, while nonmuscle myosin is required for coordinated translation of a1(I) and a2(I) mRNAs. When nonmuscle myosin filaments are disrupted, collagen mRNAs fail to co-localize to discrete sites of their translation and the cells can secrete only homotrimers of a1(I) polypeptides. This suggests that for the proper assembly of type I collagen, collagen mRNAs must be co-localized and translated in coordination. On the other hand, Serine-Threonine kinase Receptor Associated Protein (STRAP) is recruited to restrict random translation of collagen mRNAs. Like RHA, STRAP is tethered to collagen mRNAs by interaction with LARP6. STRAP prevents unrestricted translation of collagen mRNAs by interacting with eIF4A. In the absence of STRAP eIF4A freely associates with collagen mRNAs and promotes their loading onto the polysomes. This results in overproduction of collagen polypeptides, in imbalance of synthesis of a1(I) and a2(I) polypeptides and in their inefficient assembly into collagen heterotrimer. These defects can be partially restored by supplementing STRAP. The mechanism of coordinated translation of collagen mRNAs, mediated by binding of LARP6 to 5'SL of collagen mRNAs, will be presented.

396-A The role of human RLI in cell proliferation and translational regulation

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RNase L inhibitor (RLI) belongs to the family of ABC (ATP-binding cassette) proteins, which carry out a broad range of functions. Human RLI was first characterized as an inhibitor of the antiviral 2-5A/RNase L system. The RNase L pathway, however, is only found in vertebrates, whereas RLI proteins are highly conserved from archaea to eukaryotes. Moreover, RLI is essential for the viability and development of several organisms. The central role of RLI remains therefore unexplained.

Recent studies link the role of RLI to several stages of eukaryotic translation, including initiation, termination and ribosome recycling. RLI has also been found to interact with a number of translational factors.

In this study, we show that human RLI downregulation has a strong effect on cultured cell proliferation, but does not significantly affect the total protein synthesis. We demonstrate the interaction of human RLI with some translation initiation factors, which are also essential for cell cycle regulation. We finally suggest that this interaction might be essential for the translational regulation of specific proteins involved in the cell cycle rather than for general translation.

399-A Tdrd7, a RNA binding protein, acts to restrict Epha2 protein synthesis in space and time during lens development

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The formation of tightly controlled cell-cell adhesions and adherens junctions with each other enables lens fiber cells to adopt a highly ordered structure that allows for the optical transparency of the mature ocular lens. The precise spatial and temporal regulation of mRNA and local protein synthesis is essential for establishing functional compartments within eukaryotic cells, but whether such a process occurs during lens development is unclear. We report here that Tdrd7, a Tudor domain-containing RNA binding protein, is required for maintaining lens fiber cell morphology and cell-cell interactions. Lens fiber cells in cross section adopt a regular hexagonal pattern. In contrast, lens fiber cells in *Tdrd7* mutant mice appear irregular in cross section with blebbing of the cell membrane. These phenotypes resemble those observed in the lenses of mice lacking Epha2, a receptor tyrosine kinase (RTK) in the Eph-Ephrin signaling pathway that can mediate diverse processes in development. Tdrd7 expression is spatially and temporal regulated during lens development. Tdrd7 is initially expressed in distinct cytoplasmic granules in lens fiber cells at the onset of lens vesicle formation, and then shifts to nuclei when lens fiber cells differentiation commences. Previous RNA-immunoprecipitation (RIP) experiments by us have shown that Tdrd7 can bind Epha2 mRNA. Examination of Tdrd7 null lenses shows decreased Epha2 protein but not mRNA. Moreover, Epha2, which is localized primarily to the short ends of fiber cells, is mislocalized in the Tdrd7-null lens, suggesting that Tdrd7 acts to help restrict Epha2 protein synthesis in time and space.

3' end processing

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 402 A – 414 A
Location: Main Hallway & Sanada Foyer

402 A **Assessing the "geometry" of the TRAMP and exosome complexes.**
405 A **Shifting targets: microRNA variants and alternative polyadenylation in cardiac hypertrophy**
408 A **FUS protein interacts with U7 snRNP and plays a role in replication-dependant histone genes expression**
411 A **A search for new factors involved in 3' end processing of histone pre-mRNAs: proteins interacting with a complex of the conserved stem-loop and the Stem-Loop Binding Protein (SLBP) in Drosophila.**
414 A **FPA, a regulator of alternative polyadenylation, is closely associated with cleavage and polyadenylation factors in vivo**

402-A Assessing the “geometry” of the TRAMP and exosome complexes.

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The exosome complex plays major roles in RNA 3'->5' processing and surveillance activities. The TRAMP polyadenylation complexes are major cofactors for the nuclear exosome and play key roles in nuclear RNA quality control. The TRAMP complexes include the RNA helicase Mtr4 together with a Zn-knuckle RNA binding protein (Air1 or Air 2) and a poly(A) polymerase (Trf4 or Tr5). However, Mtr4 is an essential exosome cofactor of all its characterized nuclear RNA degradation and processing activities, whereas only surveillance activities are known to require the other TRAMP components. Recent structural analyses of Mtr4 have identified an "Arch" domain, which acts independently of the helicase activity and is required to stimulate exosome function *in vivo* and *in vitro*. An *in vivo* crosslinking technique (CRAC) was used to compare RNA targets of intact Mtr4 and an Mtr4-Archless construct, lacking the Arch domain. Substantial differences between RNA species associated with the two constructs were revealed. In particular, Mtr4-Arch shows reduced targeting of pre-rRNA species, consistent with observed defects in pre-rRNA processing and growth. In addition, the fraction of Mtr4-associated RNA sequences that carry TRAMP-generated, non-encoded oligo(A) tails was lower in strains lacking Arch domain. This is consistent with the reported role for Mtr4 in the regulation of oligoadenylation of target RNAs by the TRAMP complex.

In vitro functional and structural analyses show that RNA is threaded through the central channel of the exosome. However, it is also feasible that substrates could be "docked" directly to the ribonucleases Rrp44 and Rrp6. We have proposed that highly structured RNAs may be docked, rather than threaded through the lumen of the exosome core structure, and that this might help distinguish RNAs targeted for maturation or decay. To assess how Mtr4 affects the interaction of the core exosome with these different substrate classes, we performed CRAC on the exonuclease Rrp44 (WT and catalytic mutant) in strains expressing Mtr4-Archless and in strains with mutation that occlude the RNA channel within the exosome. High-throughput sequencing is currently being carried out. These analyses should clarify the role of Mtr4 and the Arch domain in substrate recruitment, exosome activation, and mode of degradation.

405-A Shifting targets: microRNA variants and alternative polyadenylation in cardiac hypertrophy

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Emerging findings indicate that cells can produce both miRNAs and their mRNA targets in multiple processing variants as a means to increase the complexity of miRNA-mediated control in a tissue and developmental stage-specific manner. MiRNAs play critical roles in the heart, and we hypothesise that during the cardiac hypertrophic response there are changes to both miRNA processing and mRNA 3' polyadenylation site selection, which will alter miRNA/mRNA interactions. Transverse Aortic Constriction (TAC), a model of left ventricular hypertrophy, was used to obtain pre-hypertrophic and hypertrophic cardiomyocytes. TAC led to pressure overload and left ventricular hypertrophy, characterised by an increase in left ventricular weight and induction of hypertrophic markers.

RNA was then extracted from purified cardiomyocytes for next-generation sequencing of small RNAs and mRNA 3' ends. Several miRNAs were deregulated prior to the development of hypertrophy and in the hypertrophic hearts. Processing variants, such as isomiRs and unexpected arm bias, of key cardiac miRNAs were also identified that could potentially alter their targeting specificity. Furthermore, numerous mRNAs encoding important cardiac functions are subjected to alternative polyadenylation that alters the length of the 3'UTR. The 3'UTR length changes may alter the extent to which miRNAs can regulate these mRNAs. The sequencing has produced global information on expression changes to both miRNA sequence and mRNA 3'UTR lengths, allowing us to form a systems level understanding of miRNA-regulation during cardiac hypertrophy.

The realisation that cardiac miRNAs and their targets exist as currently under-appreciated variants with potentially complex effects on target specificities has important implications for the role of miRNAs in cardiac disease.

408-A FUS protein interacts with U7 snRNP and plays a role in replication-dependant histone genes expression

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The U7 small nuclear ribonucleoprotein (U7 snRNP) is an essential factor mediating the unique 3'end processing of non-polyadenylated, replication-dependent histone mRNAs in metazoans. These histone genes expression and processing of their transcripts are cell cycle-regulated mechanisms that recruit a number of specific proteins as well as common factors required for expression and maturation of polyadenylated mRNAs. However, despite all the knowledge we have so far, there are still gaps in understanding of core histone RNA 3' end processing, its coupling to transcription and regulation during cell cycle. To further elucidate this phenomena we used affinity chromatography based on tagged version of U7 snRNA molecule to identify proteins associated with U7 snRNP/U7 snRNA that could be potentially involved in core histone genes expression in human cells.

Mass spectrometric analysis of affinity-purified fraction revealed, among others, multifunctional RNA/DNA-binding protein FUS/TLS (fused in sarcoma/translocated in liposarcoma) as a new factor interacting with U7 snRNA/RNP. Co-immunoprecipitation and RIP experiments confirmed the binding between FUS and the U7 RNA/snRNP. Interestingly, FUS:U7 snRNA interaction seems to be activated in S phase where the core histone genes are expressed. Moreover, FUS co-fractionates in 10-50% continuous glycerol gradient with other factors involved in histone pre-mRNAs 3'end processing. However, this unique 3'end maturation was not disturbed upon FUS knockdown. Instead, we found that FUS depletion leads to a de-regulation of expression from selected histone promoters, suggesting that FUS is rather involved in regulation of core histone genes transcription. Thus, FUS bound to U7 snRNP can play a role in coupling between transcription and 3'end processing of replication dependant histone mRNAs.

411-A A search for new factors involved in 3’ end processing of histone pre-mRNAs: proteins interacting with a complex of the conserved stem-loop and the Stem-Loop Binding Protein (SLBP) in Drosophila.

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3' end processing of animal replication-dependent histone pre-mRNAs occurs through a single-step endonucleolytic cleavage that is not followed by polyadenylation. Cleavage depends on two cis-elements in histone pre-mRNA: a highly conserved stem-loop structure (SL) and a variable purine-sequence, the Histone Downstream Element (HDE). The SL interacts with the Stem-Loop Binding Protein, whereas the HDE is a binding site for U7 snRNP. We recently showed that U7 snRNP in addition to the U7-specific Sm ring and U7 snRNA contains FLASH and a number of polyadenylation factors, including the CPSF73 endonuclease. Mammalian histone pre-mRNAs are cleaved by this enzyme at a fixed distance from the HDE. SLBP and the SL structure are dispensable for processing, although they have a stimulatory effect by stabilizing U7 snRNP on histone pre-mRNA. This indicates that the mammalian U7 snRNP alone can recruit the entire machinery required for processing. In contrast, cleavage of *Drosophila* histone pre-mRNAs depends on both the SLBP and the U7 snRNP and invariably occurs 4 nucleotides after the stem. In *Drosophila* nuclear extracts, the U7 snRNP containing CPSF73 and other polyadenylation factors is efficiently recruited to the HDE even in the absence of SLBP. Thus, *Drosophila* SLBP must function in processing by recruiting an essential factor that in turn may interact with the machinery contributed by U7 snRNP. We used *Drosophila* histone pre-mRNA prebound to recombinant SLBP to purify proteins from *Drosophila* nuclear extracts that interact with the complex but not with the histone pre-mRNA lacking SLBP. Mass spectrometry identified three proteins that fit these criteria: NOT1, a DHX-type helicase and the methyltransferase responsible for modifying the 2' hydroxyl of the cap. Importantly, these three proteins do not interact with the complex of SLBP and the SL followed by 4 only nucleotides, suggesting that they are involved in 3' end processing of histone pre-mRNAs rather than in processes operating on mature histone mRNA. We identified a different complex that interacts with the 3' end of mature histone mRNA. This complex consists of multiple aminoacyl-tRNA synthetases and binds to the terminal portion of the SL in a manner independent of binding of SLBP. Potential roles of proteins that interact with the SL in histone pre-mRNA and mRNA in 3' end processing and post-processing events and their precise mode of binding in the presence and absence of SLBP are being currently investigated.

414-A FPA, a regulator of alternative polyadenylation, is closely associated with cleavage and polyadenylation factors in vivo

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Alternative cleavage and polyadenylation influences the coding and regulatory potential of mRNA and where transcription termination occurs. Although widespread, few regulators of this process are known. First identified because of its role in flower development, the *Arabidopsis thaliana* SPEN family protein FPA is a rare example of a *trans*-acting regulator of poly(A) site choice (Hornyik et al., 2010). The characterization of FPA therefore provides an opportunity to reveal novel mechanisms by which poly(A) site selection can be controlled. Here we show that FPA is closely associated with core components of the cleavage and polyadenylation apparatus *in vivo*.

In order to understand how FPA might function, we developed a novel proteomics procedure based on the cross-linking of native protein interactions in living cells using formaldehyde. We then used statistical analysis of multiple biological replicates to compare proteins co-purified with FPA and the well-studied splicing factor U2B "as a control. We found that U2B" associates with known components of the U2snRNP and spliceosome as expected, but found little overlap between these proteins and those associated with FPA. Most proteins associated with FPA had domains connected to RNA processing and several had previously been identified as components of the same genetic pathway controlling flower development as FPA. Strikingly, almost all core components of the cleavage and polyadenylation machinery co-purified with FPA. We have substantiated these data with genetic analyses, revealing for example, that the function of FPA in promoting flower development requires an Arabidopsis protein related to the core cleavage, polyadenylation and termination factor Pcf11. Experiments designed to identify which of these co-purified proteins FPA interacts with directly are now in progress.

These findings establish a simple, generally useful procedure for label-free *in vivo* proteomic analysis of proteins involved in RNA processing. In addition, they suggest that the mechanism by which FPA regulates poly(A) site choice involves close, and possibly direct, association with the core cleavage and polyadenylation machinery.

RNA Turnover

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 417 A – 441 A
Location: Main Hallway & Sanada Foyer

417 A **Molecular genetic exploration of the yeast DEAH helicase, Mtr4, arch domain**
420 A **Adipogenesis is efficiently regulated by SMG1 via staufen1-mediated mRNA decay**
423 A **Control of mRNA decay by Puf proteins regulates ribosome biogenesis**
426 A **Poly(A)-specific ribonuclease (PARN): Mechanisms of processivity and catalysis**
429 A **RT-qPCR Reference Genes and Potassium Stress Responses in the Haloarchaeon, Haloarcula marismortui**
432 A **High-resolution characterization of regulatory sequences within a mammalian 3' UTR**
435 A **The mRNA quality control factors Ski7 and Hbs1 evolved from an alternatively spliced gene that produced Ski7-like and Hbs1-like proteins.**
438 A **A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy**
441 A **Acetylation of CAF1a and BTG2 accelerates general mRNA degradation**
444 A **Withdrawn**

417-A Molecular genetic exploration of the yeast DEAH helicase, Mtr4, arch domain

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[1]Marquette University

Our interest in understanding how RNAs are recognized and tagged for degradation by the yeast TRAMP complex led us to explore the role of the Mtr4 arch domain in how it functions as an enzyme and a member of the TRAMP complex. Mutations in the Mtr4 arch domain were identified using a genetic screen to uncover mutations in the arch domain and c-terminus that inhibit the ability of Mtr4 to support degradation of hypomodified tRNAiMet. From this screen, 40 independent mutants were identified that had either one or more mutations in the arch domain. Six mutants within the arch domain and two mutants in the c-terminus, each with the strongest inhibitory effect on Mtr4 were chosen for further characterization.

Recombinant Mtr4 proteins and each mutant were expressed and purified from E. coli and tested for ATPase activity in the presence and absence of total tRNA and synthetic RNAs of 108 nt and 76 nt and predicted to have little to no structure were used in stimulating Mtr4 enzymatic activity. Enzymatic assays run in triplicate were used to determine the Km for ATP, Kapp for RNA and Vmax for RNAs. Our analysis demonstrated that total E coli tRNA and synthetic yeast tRNAiMet are poor at stimulating Mtr4 ATPase activity. The predicted unstructure RNAs stimulated Mtr4 ATPase activity much more robustly than did tRNA as we would have expected based on ours and other peoples work.

420-A Adipogenesis is efficiently regulated by SMG1 via staufen1-mediated mRNA decay

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Suppressor of morphogenesis in genitalia 1 (SMG1), a member of the phosphatidylinositol 3-kinase-related kinases family, is involved in nonsense-mediated mRNA decay (NMD). SMG1 phosphorylates Upf1, a key NMD factor. Subsequently, hyperphosphorylated Upf1 associates with SMG5-7 or proline-rich nuclear receptor coregulatory protein (PNRC2) to elicit rapid mRNA degradation. Upf1 is also known to be involved in staufen 1 (Stau1)-mediated mRNA decay (SMD), closely related to NMD. However, the biological and molecular roles of SMG1 in SMD remain unknown. Here, we provide evidence that SMG1 is involved in SMD. SMG1 is complexed with SMD factors and overexpression of a kinase-inactive mutant of SMG1 inhibits SMD efficiency. Accordingly, the cellular localization of Stau1 into processing bodies is dependent on the level of Upf1 phosphorylation. We also find that the level of SMG1 increases during adipogenesis and downregulation of SMG1 delays efficient adipogenesis, suggesting the functional involvement of SMG1 in adipogenesis via SMD.

423-A Control of mRNA decay by Puf proteins regulates ribosome biogenesis

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The Puf family of eukaryotic RNA-binding proteins plays important roles in stem cell maintenance, cell development, and differentiation by binding conserved elements within the 3' UTR of target mRNAs, typically resulting in mRNA degradation and/or translational repression. Puf proteins are characterized by their repeat domains composed of eight tandem repeats of ~36 amino acids, whereby each repeat contacts one base within an 8-10 nt target sequence containing a conserved UGU. Once bound to an mRNA target, Puf proteins elicit RNA repression by inhibition of cap-binding events or recruitment of mRNA decay factors. The yeast *Saccharomyces cerevisiae* expresses six Puf proteins, and hundreds of candidate mRNA targets of these Pufs have been predicted from physical association or bioinformatic screens. We have focused on understanding the mechanistic roles of Pufs in regulating such target mRNAs. One of our studies analyzed several of the predicted Puf4p targets that had the highest conservation of consensus binding sequence for Puf4p, and are all involved in various steps of ribosome biogenesis. Analysis of target mRNA steady state levels in wild-type versus *PUF4?* strains revealed only small differences between the two strains, which prompted us to examine these mRNA targets in other PUF deletion strains. Our data demonstrate that regulation of all the targets examined is controlled by the combination of Puf4p and Puf5p, suggesting a "partial redundancy" of these two Puf proteins. Over-expression of Puf4p causes delays in processing of the rRNA 35S precursor. Puf4p also appears to play a role in the export of pre-ribosomal subunits. Together, the data suggests that Puf proteins play a global role in ribosome biogenesis through the decay of their target mRNAs.

426-A Poly(A)-specific ribonuclease (PARN): Mechanisms of processivity and catalysis

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Poly(A)-specific ribonuclease (PARN) is a divalent metal-ion dependent poly(A)-specific, processive and cap-interacting 3'-5' exoribonuclease that efficiently degrades poly(A) tails of eukaryotic mRNAs. PARN is unique among the poly(A) degrading nucleases, being the only one that has the capacity to directly interact during poly(A) hydrolysis with both the m^7G-cap structure and the poly(A) tail of the mRNA. On the basis of biochemical and structural evidence we present and discuss a working model for PARN action. The model defines two alternating reaction steps, a translocation event that pushes the scissile bond of the poly(A) substrate into the catalytic center of the active site followed by a hydrolytic event that cleaves the scissile bond. The two reaction steps are repeated and in each cycle one AMP residue is released. The reaction cycle depends on the coordinated movements of the RNA recognition motif (RRM), which pushes the substrate into the active site, and a catalytically essential His residue of the catalytic center. The model also provides a plausible mechanistic framework for how the m^7G-cap structure could allosterically affect the hydrolytic activity of PARN. Structural and functional data supporting the model will be presented. Significantly, we have found that divalent metal ions are required for both hydrolysis and substrate translocation in the active site. Our data imply that three divalent metal ions are required for proper action. Two ions participate in hydrolysis while the third plays a key role during translocation. The generality of this proposal in relationship to other processive enzymes participating in cleavage or formation of phosphodiester bonds will be discussed.

429-A RT-qPCR Reference Genes and Potassium Stress Responses in the Haloarchaeon, Haloarcula marismortui

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Potassium ion transport is of interest in many systems as K+ concentrations are thought to be used to maintain or change a membrane potential. In halophiles, such as *Haloarcula marismortui*, intracellular potassium ion (K^+) concentration is postulated to be a mechanism for balancing the osmotic pressure experienced in a hyper-saline environment[1], and unlike most organisms, these concentrations have been reported to be in excess of 3M [2,3]. We are interested in the dependence of these halophiles upon the external and internal K+ concentration with respect to osmotic stress.

We demonstrate that cellular generation time for the halophile varies with extracellular K^+ concentration, and growth occurs on alternative monovalent ions including lithium, rubidium, and caesium at concentrations equivalent to the observed K^+ optima. We have also shown that intracellular ion concentrations correlate to ion concentrations in the media. Our results suggest *Har. marismortui* is both highly selective for, and an excellent scavenger of K^+.

In light of this information, we used qRT-PCR to analyze the change in expression of any postulated potassium transport systems. Genome analysis[5] indicates the possible existence of three separate potassium channel/transport systems in *Har. marismortui*, Trk, MthK and Pch. These are othologous to the *Escherichia coli* K-transport systems KcsA and Kch. TrkA and MthK are both KcsA orthologs, the is the constitutive K channel in *E. coli*[6], while Pch is the ortholog of the Kch channel. Kch is similar to the voltage-gated K channels (Kv channels) in eukaryotic K-channels as it has a similar P-loop region, even though specific sequence identity is low, and is expressed under low K+ conditions in *E. coli* as it has an active transport mechanism[6]. As prior archaeal qRT-PCR studies appear to use only a single reference gene, we assessed the stability of several canadidate genes in order to meet the minimum of 3 reference genes as recommended by the MIQE Guidelines[4]. Using these novel references, expression of potassium channel genes was examined in cellular shock (5 min, 2 h) and stable growth (2 week) conditions at each of the analyzed K+ conditions.

References:

1. Oren, A., *et al* (*1990*) *Int. J. Syst. Bacteriol.* **40**, 209-210.
2. Christian, J. H. B. & Waltho, J. A. (1962) *Biochim Biophys Acta* **65**, 506-508.
3. Ginzburg, M., *et al* (1970) *J Gen Physiol* **55**, 187-207.
4. Bustin, S. A., *et al*. (2009) *Clinical Chemistry* **55**, 611-622.
5. Baliga N.S. *et al* (2004) *Genome Res* **14**:2221-2234.
6. Loukin, S. H. *et al* (2005) *J. Gen. Physiol*. **125**, 521-527.
7. Kuo, M. M-C., *et al* (2003) *EMBO Journal* **22**, 4049-4058.

432-A High-resolution characterization of regulatory sequences within a mammalian 3' UTR

Katla Kristjansdottir[1], *RaeAnna Wilson*[1], *Elizabeth Fogarty*[1], *Andrew Grimson*[1]

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Many important gene regulatory events occur post-transcriptionally, typically by regulation of mRNA translation and decay rates. Post-transcriptional regulatory events are usually controlled by *trans*-acting factors (such as microRNAs) that bind to *cis*-acting sequence elements (*cis*-elements), which are often located within the 3' untranslated regions (3' UTRs) of mRNAs. Importantly, previous analyses suggest that typical mammalian 3' UTRs contain many different *cis*-elements and that certain cis-elements can interact in synergistic or inhibitory ways. While many *cis*-elements have been identified, most of our current understanding of their function is based on analysis of elements in isolation rather than in combination with other elements. The work presented aims to better understand the range of regulatory sequences within a 3' UTR and how sequences within 3' UTRs interact with one another. Towards that goal, I have been studying in detail the 3' UTR of the mouse HMGA2 gene, which is known to direct substantial post-transcriptional regulation of its mRNA. The regulatory information within this 3' UTR is particularly important, since HMGA2 over-expression due to 3' UTR truncations is found in many types of cancer and is linked with a poor prognosis.

To identify regions that confer regulation, I used reporter assays to assess the regulatory information within 100 nucleotide (nt) segments (100mers) tiled across the HMGA2 UTR, generating a high-resolution *cis*-regulation map of the UTR. This map shows several new regions of regulation beyond those previously described. For each strongly regulating 100mer, I am currently identifying the specific *cis*-elements responsible, which will be critical in identifying the *trans*-factors involved. To understand how interactions between *cis*-elements contribute to regulation, I also measured 200 nt segments (200mers) tiled across the 3' UTR. If elements within two adjacent 100mers do not interact, we expect the regulatory potential of the corresponding 200mer to be recapitulated by the regulatory potential of the 100mers. By looking for 200mers that fail to be recapitulated in this manner, I have been able to identify four regions that contain *cis*-elements that may be interacting. I am currently working on characterizing those interactions towards a mechanistic understanding of their function. Getting a complete picture of the *cis*-regulatory information within 3' UTRs, and how the *cis*-elements can interact to control the mRNA, is an important step towards a fuller understanding of post-transcriptional gene regulation.

435-A The mRNA quality control factors Ski7 and Hbs1 evolved from an alternatively spliced gene that produced Ski7-like and Hbs1-like proteins.

Alexandra N. Marshall[1], *Maria Camila Montealegre*[1], *Claudia Jiménez-López*[1], *Michael C. Lorenz*[1], *Ambro van Hoof*[1]
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One major function of mRNA degradation is to maintain fidelity of gene expression by specifically targeting aberrant mRNAs for rapid degradation. Two *Saccharomyces cerevisiae* paralogs play central roles in this process. The recognition and degradation of mRNAs that lack a stop codon requires Ski7 and the RNA exosome, while the recognition and degradation of mRNAs with stalled ribosomes in the middle of an ORF requires Hbs1 and an unknown endonuclease. In addition to its role in recognizing nonstop mRNAs, Ski7 has a second role as a cytoplasmic exosome cofactor. Ski7 and Hbs1 are paralogs that arose in budding yeast about 100 million years ago, and thus most other eukaryotes only contain one corresponding gene. How other eukaryotes recognize and degrade nonstop and no-go mRNAs, and whether they contain a Ski7-like exosome cofactor was not fully understood.

Lachancea kluyveri is closely related to budding yeast, but like other eukaryotes has only one *SKI7/HBS1* gene. rt-PCR, RNA-seq and Western blot analysis show that this one gene encodes two distinct proteins through alternative splicing. Furthermore, the longer splice isoform functions as Ski7, while the shorter splice isoform functions as Hbs1. Thus, the expression of two distinct proteins to recognize nonstop and no-go mRNAs is more widespread than anticipated.

While alternative splicing is much less common in fungi than in mammals, the alternative splicing of *SKI7/HBS1* is conserved in both the ascomycetes and the basidiomycetes as shown by rt-PCR and bioinformatics analysis. Although alternative splicing of *SKI7/HBS1* is conserved, the exact mechanism has changed several times during fungal evolution, such that both alternative 3' splice sites, alternative first exons, and likely other mechanisms are used. Strikingly, other than *S. cerevisiae* and its close relatives, the only other fungi where we failed to detect alternative splicing are in the *Schizosaccharomyces* genus. While this genus is very distantly related to *S. cerevisiae*, it also contains an uncharacterized *SKI7* gene in addition to its canonical *HBS1* gene. This comparison of splicing strategies in diverse fungi provides a model to understand the evolutionary changes in alternative splicing.

The human genome also contains only a single ortholog whose major splicing isoform resembles Hbs1, both in sequence and biochemical activity. However, the human gene is also alternatively spliced, and our results suggest that the alternatively spliced isoform functions as the missing Ski7-like cytoplasmic exosome cofactor.

438-A A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is amongst the leading genetic causes of infant deaths and is characterized by specific degeneration and loss of spinal cord motoneurons. It is caused by the disruption of the "survival of motor neurons" gene (SMN1), which plays an essential role in the cytoplasmic assembly of core snRNPs. To date, we still do not understand why the motoneurons are the most affected cell type in response to a deficiency in this protein. It has been proposed that motoneurons are more sensitive to low snRNPs levels and splicing defects. Alternatively, it has also been proposed that SMN may have a distinct function specifically in motoneurons. We recently uncovered that SMN normally represses CARM1 translation, thus leading to an increase of CARM1 expression in SMA. Since our results obtained for the past 4 years strongly suggest a key role of CARM1 in the etiology of SMA, we decided to investigate the impact of an increase of CARM1 in the disease. Amongst the different cellular roles of CARM1, we decided to focus on the relationship between the massive alteration of splicing in SMA and the fact that CARM1 can regulate this mechanism. Thus, our objective is to identify splicing targets that are misregulated by CARM1 in the pathology.

While pursuing splicing targets regulated by CARM1, we uncovered a completely novel function for CARM1 as a regulator of nonsense-mediated mRNA decay (NMD). Interestingly, in our preliminary results we identified USPL1, as a target regulated by CARM1. USPL1 is a gene which has been found to be mis-spliced in a number of difference SMA cell culture and in vivo models. This gene can generate two transcripts including or not alternative exon 2. In SMA, it was reported that the splicing variant containing exon 2 is enriched. Upon analysis of the sequences of these two USPL1 splicing isoforms, we observed that the transcript without the exon 2 in fact matched the criteria to be a NMD-regulated target. To test this hypothesis, we decided to inhibit the NMD pathway by using either drugs or a RNAi strategy against UPF1 (the main effector of NMD). Thanks to these strategies, we were able to confirm that this variant was a NMD target. Afterwards, to confirm a role of CARM1 in the NMD mechanism, we used a well-accepted NMD reporter assay which allowed us to validate our hypothesis. Furthermore, by using co-immunoprecipitation experiments, we observed an interaction between CARM1 and both UPF1 and UPF2, but not with UPF3 or components of the EJC, suggesting CARM1 is not recruited co-transcriptionally to NND transcripts and may actually play a direct role in the early activating steps of NMD. Finally, our preliminary screen has identified a few additional NMD targets regulated by CARM1, that are also misregulated in SMA. Altogether, identification of the full spectrum activities of CARM1 may provide crucial insights into our understanding of the etiology of SMA.

441-A Acetylation of CAF1a and BTG2 accelerates general mRNA degradation

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The half-life of most mRNAs is determined by the rate of poly(A) tail shortening. In metazoans, two deadenylases, CAF1a and CAF1b, play a major role in removing poly(A) tails in the cytoplasm, thereby initiating degradation of mRNAs. The CAF1 enzymes are integral components of the CCR4-CAF1-NOT complex, which is actively recruited to mRNAs destined for degradation. In addition, CAF1a and CAF1b interact with the TOB/BTG family of proteins, which promote the activity of CAF1 enzymes. Given the central importance of CAF1 deadenylases in mRNA turnover, we examined whether their activity is regulated.

Here we report that hyperacetylation strongly promotes the interaction between human CAF1a and BTG2 *in-vivo*. In accordance with this finding, hyperacetylation accelerated degradation of stable mRNAs. Hyperacetylation also caused a general loss of poly-A tails, pointing towards a general activation of deadenylation. Using an anti acetyl-lysine antibody on purified proteins, we found that both CAF1a and BTG2 are directly acetylated. By testing candidate enzymes, we were able to identify two acetyl transferases that promote acetylation of CAF1a and BTG2, as well as a deacetylase that removes lysine acetylation on both proteins.

Mass spectrometry revealed acetylated lysine residues on both CAF1a and BTG2. Mutation of two lysine residues in BTG2 was sufficient to abolish acetylation. Importantly, the same mutation also prevented proteasome-dependent degradation of BTG2, indicating that acetylation stabilizes BTG2. The analysis of reporter mRNA degradation further suggested that the activities of CAF1 and BTG2 are enhanced by hyperacetylation. Taken together, our data show for the first time that acetylation plays an important role in regulating posttranscriptional gene expression by promoting the general turnover of mRNAs.

444-A Withdrawn

RNA Editing and Modification

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 447 A – 471 A
Location: Main Hallway & Sanada Foyer

447 A **Activation of RNA cap methylation by CDK1**
450 A **A Hundred million sites located in majority of human genes are subject to A-to-I RNA editing**
453 A **Identifying the function of highly conserved residues for pseudouridine formation through a combination of in silico and in vitro studies**
456 A **Conserved circuitry in eukaryotes for crucial Trm7 modifications of the tRNA anticodon loop**
459 A **2´-SCF3 Modified Pyrimidine Nucleosides as Labels for Probing RNA Structure and Function by 19F NMR Spectroscopy**
462 A **Structural features of Cbf5 and guide RNA involved in the functions of archaeal box H/ACA RNP complexes**
465 A **tRNA wobble uridine hypomodification decreases the decoding efficiency of cognate codons in vivo**
468 A **Differential expression of Human ADAT subunits**
471 A **Impact of inverted SINEs on gene expression**

447-A Activation of RNA cap methylation by CDK1

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Eukaryotic RNA polymerase II transcripts, such as mRNA, are modified by the addition of a 7-methylguanosine cap which is essential for gene expression and cell viability. The methyl cap stabilises mRNA by protecting it from 5' to 3' exoribonucleolytic degradation, and interacts with the cap-binding complex (CBC) and the eukaryotic initiation factor 4E (eIF4E), thus promoting transcription, splicing, polyadenylation, nuclear export and translation initiation. In mammals, 7-methylguanosine cap formation is catalysed by the capping enzyme (RNGTT) which adds a guanosine cap to the nascent mRNA and the RNA guanine-7 methyltransferase complex (RNMT-RAM) that methylates the cap. The catalytic domain of the methyltransferase complex resides on RNMT whereas RAM has a regulatory effect.

Recent work demonstrated that methyl cap formation is a regulated process. The transcription factors E2F1 and c-Myc can increase 7-methylguanosine levels of specific transcripts. Futhermore, methyl cap synthesis can be regulated under normal and stress conditions such as amino acid starvation.

In order to better understand the molecular mechanisms that regulate cap methylation in human cells we investigated RNMT post-translational modifications. Analysis by orthophosphate labelling and mass spectrometry revealed that RNMT is phosphorylated. We identified the predominant phosphorylation site and demonstrated that Cyclin-dependent kinase 1 (CDK1/Cyclin B) is responsible for RNMT phosphorylation during G2/M phase of the cell cycle. Interestingly, RNMT phosphorylation increases cap methyltransferase activity in vitro and loss of this phosphorylation site reduces cell proliferation. Preliminary data suggests that RNMT phosphorylation regulates gene expression in gene-specific manner.

Currently, we are investigating why RNMT is phosphorylated during G2/M phase and why RNMT phosphorylation regulates specific transcripts.

450-A A Hundred million sites located in majority of human genes are subject to A-to-I RNA editing

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

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RNA molecules carry the information encoded in the genome and reflect its content. Adenosine-to-inosine modification of RNA molecules (A-to-I RNA editing) by ADAR proteins converts a genomically encoded adenosine (A) into inosine (I). It is known that most RNA editing in human take place in the primate specific Alu sequences but the scope of this phenomenon and its effect of transcriptome diversity is not clear yet. Here we show, by analyzing large-scale RNA-seq data and by performing ultra-deep

sequencing of selected Alu sequences, that the scope of editing is much larger than was anticipated. More than 700,000 Alu repeats can form dsRNA structures, and virtually all adenosines within these Alu repeats undergo A-to-I editing to some extent. Moreover, we observe editing of transcripts resulting from residual anti-sense expression, doubling the number of edited sites in the human genome. The total number is thus estimated to exceed 100 million sites. Our ultra-high coverage enables us to probe editing levels that span a wide range, with few sites being fully converted while most sites exhibit low levels (<1%). Our finding set the stage for exploring how this primate-specific diversification of the transcriptome is utilized.

453-A Identifying the function of highly conserved residues for pseudouridine formation through a combination of in silico and in vitro studies

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Pseudouridine is the most abundant post-transcriptional RNA modification and is formed through the isomerization of uridine to pseudouridine by enzymes known as pseudouridine synthases. These enzymes share a common catalytic domain and active site structure including a strictly conserved aspartate residue that is essential for catalysis. However, the exact mechanism of pseudouridine formation is still unknown. Here, a combination of *in silico* and *in vitro* studies was used to identify residues involved in catalysis in order to better understand the catalytic mechanism of pseudouridine synthases. First, molecular dynamics simulations of the *E. coli* pseudouridine synthase TruB, responsible for the formation of pseudouridine 55 in tRNA, were performed in the absence of substrate for 40 ns in two functionally different conformations. The simulations revealed two highly conserved residues, R181 and D90, that showed differential interaction patterns with the catalytic D48 residue. As known from crystallographic studies, R181 can form a hydrogen bond with the catalytic D48 residue. Interestingly however, R181 can also interact with the second-shell residue D90. This suggests that R181 may switch between two conformations, facilitated by D90. This switch might in turn be important for catalysis. To test this hypothesis, *in vitro* mutational studies on R181 and D90 were conducted to identify the precise roles of R181 and D90 for TruB's function. Although substrate binding was not affected by amino acid substitutions at these positions, catalysis was severely impaired. Upon changing D90 to glutamate, asparagine or alanine, the rate of pseudouridine formation decreased 30 to 600-fold, respectively. Likewise, substituting R181 with lysine reduced the catalytic rate over 2500-fold while substitutions with methionine or alanine caused a reduction of more than 5000-fold. Our findings suggest that R181 and D90 work together to position the catalytic residue D48 which is critical for efficient pseudouridine formation. Further experiments and molecular dynamics simulations are underway to identify in which step of TruB's mechanism R181 and D90 are involved. In summary, the use of molecular dynamics simulations in combination with *in vitro* assays has enabled us to identify two residues important for catalysis and will assist in determining the mechanism of pseudouridine formation that may be common to all pseudouridine synthases.

456-A Conserved circuitry in eukaryotes for crucial Trm7 modifications of the tRNA anticodon loop

Michael Guy[1], *Eric Phizicky*[1]

[1]University of Rochester Medical School

Post-transcriptional modification of the anticodon loop of tRNA is critical for translation and proper cell growth. We recently reported that lack of 2'-*O*-methylated C_{32} (Cm_{32}) and Nm_{34} in *Saccharomyces cerevisiae trm7*-? mutants causes slow growth due to reduced function of $tRNA^{Phe}$, but not of the other Trm7 substrates $tRNA^{Trp}$ and $tRNA^{Leu(UAA)}$ (Guy M. P. *et al*., (2012) *RNA*. **10**:1921-33). In addition, we described a complex circuitry for anticodon loop modification, in which Trm7 also requires its interacting partner Trm732 for Cm_{32} formation and its interacting partner Trm734 for Nm_{34} formation, and showed that Cm_{32} and Gm_{34} formation drives subsequent production of yW_{37} from m^1G_{37} on $tRNA^{Phe}$.

Available evidence suggests that the circuitry for Trm7 modification of tRNA is conserved and is important in other eukaryotes. 2'-*O*-methylation of the anticodon loop of tRNA is common to all characterized eukaryotic $tRNA^{Phe}$ and $tRNA^{Trp}$ species, and putative *TRM7*, *TRM732*, and *TRM734* orthologs are found in the vast majority of eukaryotes. Moreover, the putative human *TRM7* homolog *FTSJ1* is associated with X-linked mental retardation, and the putative *Schizosaccharomyces pombe* Trm7 homolog is reported to be essential in high throughput studies.

Consistent with a conserved circuitry and function, our results demonstrate that Trm7 and Trm732 orthologs from humans, *S. pombe*, and *Drosophila* complement the corresponding *S. cerevisiae* mutants, and in each case can function with the corresponding *S. cerevisiae* interacting partner. Furthermore, we find that *S. pombe trm732*-$Delta; and *trm734*-Δ mutants lack Cm_{32} and Gm_{34} respectively in their $tRNA^{Phe}$. Thus, we suggest that the circuitry of Trm7, Trm732, and Trm734 is conserved in eukaryotes for modification of the anticodon loop of substrate tRNAs.

459-A 2´-SCF_3 Modified Pyrimidine Nucleosides as Labels for Probing RNA Structure and Function by ^{19}F NMR Spectroscopy

Marija Košutic[1], Katja Fauster[2], Christoph Kreutz[2], Ronald Micura[2]

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The high natural abundance and intrinsic sensitivity of ^{19}F make it a perfect orthogonal NMR probe for investigating RNA and other biomolecules in *in vitro* and *in vivo* systems. Incorporation of three equivalent fluorine atoms into a biomolecule provides experimental performance at micromolar concentrations which is a significant improvement compared to the low millimolar range required for the single-atom labels. In this study, we present synthetic pathways to 2´-SCF_3 labeled uridine [1] and cytidine building blocks and their incorporation into oligoribonucleotides using the 2´-*O*-TOM-methodology for RNA solid-phase synthesis. Furthermore, the efficient synthetic access to 2'-SCF_3 modified RNA prompted us to evaluate the new label in ^{19}F NMR applications. Here, we demonstrate three examples: 1) probing of the secondary structure of bistable RNA; 2) verification of RNA–protein interactions; and 3) attesting a rationally designed riboswitch module.

[1] Fauster, K., Kreutz, C., & Micura, R. (2012). 2'-SCF_3 Uridine –A Powerful Label for Probing Structure and Function of RNA by ^{19}F NMR Spectroscopy. Angewandte Chemie International Edition, 51(52), 13080-13084.

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462-A Structural features of Cbf5 and guide RNA involved in the functions of archaeal box H/ACA RNP complexes

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Pseudouridine (Ψ), the C-5 ribosyl isomer of uridine, is commonly found at several positions of stable RNAs of all organisms. In addition to single or multi-site specific protein-only Ψ synthases, Eukarya and Archaea have specific ribonucleoprotein (RNP) complexes that can also produce Ψ at many sites of different cellular RNAs. Each complex contains a distinct box H/ACA guide RNA and four core proteins, Cbf5 (NAP57 or dyskerin in mammals), Gar1, Nop10 and L7Ae (Nhp2 in Eukarya). Cbf5 is the Ψ synthase in these complexes. Here, we show that Ψs at 23S rRNA positions 1940, 1942 and 2605 of *Haloferax volcanii* (*E. coli* positions 1915, 1917 and 2572) are absent in both Cbf5 and a double stem-loop box H/ACA RNA deleted strains. Plasmid borne copies of *cbf5* and the RNA can rescue the syntheses of these Ψs. Based on *Pyrococcous furiosus* crystal structure (PDB code 2EY4) we identified several potential residues and structures in *H. volcanii* Cbf5, which play important role in pseudouridylation. We mutated these structures and determined their in vivo effects towards Ψ production at the three rRNA positions. Mutations of some residues that are conserved in all Ψ synthases, and certain residues in the thumb loop resulted in absence of Ψs at all three positions. However, mutations of a few residues abolished one of the three Ψs, and some mutations showed partial ? production. Similarly, by changing a number of structures and mutating certain nucleotides in a single stem-loop box H/ACA RNA (the stem-loop responsible for Y2605 in *H. volcanii*) we show that the conserved 3' ACA (AUA here) trinucleotide is not essential for Y formation in vivo and also in vitro (using *Methanocaldococcous jannaschii* recombinant proteins). However, this trinucleotide is required for a stable binding of the Cbf5 to the guide RNA. Furthermore, a proper kink-turn with the two highly conserved G:A pairs (5' and 3') is essential for L7Ae binding as well as pseudouridylation. The guide-target pairings at both 5' and 3' sides of the Y pocket are needed for the modification. This study determines certain essential structures of both Cbf5 and box H/ACA guide RNA in Archaea that are necessary for a functional RNP formation.

465-A tRNA wobble uridine hypomodification decreases the decoding efficiency of cognate codons in vivo

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Nucleotide modifications in tRNA are ubiquitous in all domains of life and those in the tRNA anticodon are important for accurate codon recognition during translation. Thiolation at the 2-carbon (s^2) of the wobble uridine (U_{34}) base is universally conserved in three tRNA species - tE(UUC), tK(UUU), and tQ(UUG). In the cytoplasm of eukaryotes, U_{34} also carries a 5-methoxycarbonylmethyl group (mcm^5). The ELP complex is required for mcm^5 formation, while the URM1 pathway mediates 2-thiolation. Aberrant U_{34} modification is associated with diverse phenotypes such as hypersensitivity to a wide range of chemical stresses in yeast, neurological dysfunction in nematodes, and cytokinesis defects in human cells. In yeast, the phenotypes can be suppressed by overexpressing hypomodified tK(UUU) and tQ(UUG), but the underlying molecular mechanisms are unknown. To delineate them, we analyzed the *in vivo* roles of U_{34} modification in *Saccharomyces cerevisiae*. We found that the stress sensitivities of cells lacking URM1 pathway or ELP complex genes are increased in strains deleted for chaperones linked to protein synthesis. Wild-type and U_{34} modification-deficient yeast exhibited similar polysome profiles, arguing against broad defects in translation elongation in the mutant strains. Thus, we explored potential codon-specific defects in translation by ribosome profiling. This approach allowed us to quantitatively compare transcriptome-wide ribosome occupancy in wild type and a U_{34} thiolation-deficient strain (*ncs2?*) grown in rich medium or subjected to diamide-induced oxidative stress. Diamide treatment led to widespread changes in gene expression with similar magnitude in both strains, suggesting that, despite the hypersensitivity of *ncs2?* to diamide, lack of U_{34} thiolation does not compromise the cellular response to this stress. The number of transcripts with altered ribosome loading in the deletion strain, however, was larger in the expression response elicited by diamide. Strikingly, we detected significantly higher ribosome occupancy for codons read by the hypomodified tRNAs in the putative A site within RPFs from *ncs2?* yeast. This effect was discernible in both unstressed and diamide-treated cells and was accompanied by smaller increases of cognate codon occupancy upstream of the A site. Our data thus suggests that U_{34} hypomodification is associated with translational slowdown at cognate codons *in vivo* that may be intensified by combinations of these codons. We are currently investigating whether this phenomenon is conserved in *Caenorhabditis elegans* and what its impact is on protein abundance and function.

468-A Differential expression of Human ADAT subunits

Adrián Gabriel Torres[1], Adélaïde Saint-Leger[1], Francisco Miguel Torres[1], Eva Maria Novoa[1], Anna Tor[1], Lluís Ribas de Pouplana[2]
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We have reported that two specific tRNA wobble base modifications contributed to genome evolution and extant codon usage biases. We showed that, contrary to prior observations, genomic codon usage and tRNA gene frequencies correlate in Bacteria and Eukarya if these two modifications are taken into account, and that presence or absence of these modifications explains patterns of gene expression observed in previous studies. We experimentally demonstrated that human gene expression levels correlate well with genomic codon composition if these identified modifications are considered [1].

As a continuation to this work we have started to characterize the biological role of ADAT (adenosine deaminases acting on tRNA) in mammalian cells, and in particular the potential effects of variations in the levels of ADAT activity upon the human proteome. Our initial results indicate that ADAT levels do indeed vary, and that this variation is due to changes in the levels of one of the enzyme's subunits. We will discuss these results at the meeting.

471-A Impact of inverted SINEs on gene expression

Mansoureh Tajaddod[1], *Konstantin Licht*[1], *Florian Huber*[1], *Michael Jantsch*[1]

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The short interspersed elements (SINEs) comprise the largest family of repetitive elements in the mammalian genome and are enriched in gene rich regions. SINEs frequently occur within genes where they are mostly located in introns and UTRs. SINEs can have a dramatic impact on the transcriptome by several means such as repressing global transcription by impairing polymerase II activity, affecting folding and splicing, or by triggering Staufen mediated decay. In addition, SINE elements in inverted orientation can basepair and have been reported to control gene expression. As underlying mechanisms, RNA-editing, sequence specific degradation, or translational control has been discussed.

Here we aim at determining the impact of inverted, basepaired SINEs located in 3' UTRs on gene expression. So far we could show that the presence of inverted SINE in 3' UTRs can repress gene expression and reduce mRNA levels. Using knock out cells we could show that the reduced gene expression is sequence-independent and does not rely on known double-stranded RNA-dependent pathways, such as Dicer, Staufen, PKR, or ADAR.

The reduced RNA levels measured for RNAs containing basepaired double-stranded SINEs could not be correlated with an increase in mRNA decay. It therefore seems possible that inverted SINES can control the rate of RNA synthesis.

Thus, besides the previously reported nuclear retention and translational repression induced by inverted SINEs, a third mechanism may be triggered. The signals and pathways required for this repression are currently being investigated.

Splicing Mechanisms

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 474 A – 486 A
Location: Main Hallway & Sanada Foyer

474 A **Genetic analysis of the Prp28-bypass mutant reveals further insights on U1 snRNP/5' splice site interaction**
477 A **smFRET studies of U6 during spliceosome activation in budding yeast**
480 A **Prp8-substrate interactions in yeast spliceosomes**
483 A **BRAF branch point mutation confers Vemurafenib resistance**
486 A **Localization of the pre-mRNA path in the activated yeast spliceosome by immuno-EM**

474-A Genetic analysis of the Prp28-bypass mutant reveals further insights on U1 snRNP/5' splice site interaction

Shang-Lin Chang[1], *Tien-Hsien Chang*[1]

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Essential genes are mostly ancient, conserved, and indispensable components in the genome. Most of them are involved in critical cellular processes, representing the "core genome" of an organism. However, recent studies have shown that a number of essential genes can be made dispensable in the presence of specific "bypass mutations", providing nonconventional routes to understand the functions of essential genes. We have previously shown that the otherwise essential Prp28 DExD/H-box splicing factor, known to facilitate the U1/U6 switch at the 5' splice site (5'ss) during spliceosomal assembly, can be bypassed by specific alterations in U1-snRNP-related components. These alterations include specific changes in U1C, Snu71, Prp42, Cbp80, and Ynl187. Detailed analyses on one of bypass mutants, i.e., the *prp28? U1C-[L13F]* strain, suggested that weakening the U1-5'ss base pairing is likely to account for dispensing the essential Prp28. These results indicated that Prp28 counteracts the stabilizing effect of U1C. To dissect the intricate U1 snRNP/5'ss interactions, we sought to isolate dominant mutations capable of rescuing the cold-sensitive (cs) phenotype of the Prp28-bypass strain (see above), which reflects the stalling of U1 snRNP dissociation from the 5'ss at low temperature in the absence of Prp28. The isolated 24 mutations are mapped to at least three linkage groups. Cloning of the suppressor genes from two linkage groups revealed specific mutations, often locate at the conserved positions, in genes encoding U1C and Snu71. Identification of the third linkage group is underway. These data suggest U1C and Snu71 may engage specific protein-protein or RNA-protein interactions on U1 snRNP/5'ss during pre-mRNA splicing. Ongoing biochemical analyses via 4-thioU and psoralen crosslinking shall provide mechanistic insights into the roles of these U1 snRNP components, which remain largely unclear. The described approach for probing complex mechanism should be applicable to other systems in which an essential gene can be bypassed.

477-A smFRET studies of U6 during spliceosome activation in budding yeast

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Both splicing fidelity and chemistry rely on a defined series of snRNA structural rearrangements catalyzed by DExD/H helicases and regulated via other trans-acting factors. The highly conserved snRNA U6 plays a direct role in splicing catalysis. Catalysis requires the presence of a 3' U6 internal stem loop (ISL) structure that coordinates catalytically essential Mg2+ ions analogously to domain V of group II introns. To prevent aberrant ISL formation U6 conformation is very highly regulated. U6 arrives at pre-mRNA base-paired with U4 in the U4/U6-U5 tri-snRNP, which sequesters the U6 ISL, blocking premature U6 activity. U4/U6 unwinding allows for the mutually exclusive U2/U6 interactions required for catalysis to form. In yeast U4/U6 unwinding is performed by DExD/H box helicase Brr2, a component of the U5 snRNP. Intriguingly, it has been shown that, in the presence of ATP and Mg2+, Brr2 will unwind U4/U6 duplexes in purified tri-snRNP in the absence of pre-mRNA, which indicates that Brr2 activity must be carefully regulated in vivo. Previous experiments indicate that Brr2 activity is modulated by U5 component Prp8 and Snu114, however Prp8, and Snu114 are tri-snRNP components as well and must also be tightly temporally and spatially regulated. Despite extensive knowledge of the players involved, the kinetics and fundamental molecular mechanisms of U4/U6 unwinding by Brr2 and its regulation are currently unknown. This deficiency results from inherent limits to bulk experimental systems wherein the multiple reversible steps in spliceosome assembly proceed asynchronously, and the splicing reaction itself is inefficient. We are currently generating and testing reagents required to perform smFRET. We have developed a Cy3/Cy5 intramolecular U6 labeling scheme to monitor the formation of the U6 ISL, which assembles into U6 snRNPs and is capable of in vitro splicing. Using this system, we can provide fundamental kinetic and molecular information on U4/U6 unwinding, and how unwinding is regulated during spliceosome activation.

480-A Prp8-substrate interactions in yeast spliceosomes

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The U5 snRNP protein Prp8 interacts with all 3 sites of chemistry in pre-mRNA substrates and with crucial residues in U5 and U6 snRNAs. These contacts have been mapped to the central region of yeast Prp8 by site-specific RNA-protein cross-linking and cleavage at engineered protease sites [Turner et al., RNA 12: 375-386 (2006)]. We have now used cleavage at natural and engineered Methionine residues by Cyanogen Bromide to map Prp8 contacts with the spliceosome's catalytic RNA core in more detail. Spliceosomes were stalled between catalytic steps 1 and 2 by 3' splice site mutations AG>AC or AG>AdG and captured via an epitope tag on Prp19 or one of the step 2-specific splicing factors [Slu7 or Prp18]. These techniques enabled us to monitor and physically map Prp8 contacts with the intron Branch Point [BP] and the 3' Splice Site [3'-SS].

Branch Point and 3' Splice Site cross-links map between residues 1585 and 1598 in yeast Prp8. In our recent structure of Prp8 [Galej et al., Nature 493: 638-643 (2013)] this sequence is part of a highly conserved linker region between the Reverse Transcriptase [RT] and Endonuclease [En] domains. Alanine-scanning mutagenesis of this region of Prp8 produced mutants in or near the cross-linked peptide with slow growth or lethal phenotypes. Moreover splicing assays *in vitro* revealed that these mutations caused accumulation of splicing intermediates. The cross-links from 2 of the sites of splicing chemistry [BP and 3'-SS] together with the positions of numerous splice site suppressor mutations unambiguously locate the spliceosome's catalytic RNA core to a cavity formed by the RT, En and RNaseH-like domains of Prp8.

483-A BRAF branch point mutation confers Vemurafenib resistance

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The serine/threonine kinase BRAF is a proto-oncogene that acts in the MAP kinase pathway, connecting mitogen signals to transcription of proliferation genes. Constitutively activating mutations of *BRAF* are the cause of more than 60% of melanomas. The most prevalent BRAF melanoma mutation is V600E which constitutively activates downstream signaling pathways. Vemurafenib is a potent inhibitor of BRAF (V600E), however, patients rapidly develop resistance. One Vemurafenib resistance mechanism is the emergence BRAF (V600E) splicing isoform lacking exons 4 to 8 (3-9 isoform), harboring the RAS-binding domain. Here we show that a branch point (BP) mutation in intron 8 of BRAF (V600E) is the cause for the emergence of the BRAF 3-9 isoform in a subset of melanoma cells. We show that impairing BP recognition by either silencing SF3b155 or by the use of spliceostatin A promotes 3-9 usage. Using a BRAF minigene, we show that the BP mutation changes the ratio of splicing of the reporter minigene in favor of the 3-9 isoform. Similarly, increased intron 3 length favors 3-9 splicing, suggesting slower removal of intron 3 as the reason for mis-splicing. In support, slower removal of intron 3 compared to intron 4 was also found in the BP mutated melanoma cells. We identified SRp55 binding sites in the vicinity of the mutated BP and silencing of SRp55 in the BP mutant cells reduced the 3-9 splicing. These observations represent the first steps in characterizing aberrant BRAF splicing responsible for Vemurafenib resistance and they might point to a therapeutic strategy to eliminate Vemurafenib resistance.

486-A Localization of the pre-mRNA path in the activated yeast spliceosome by immuno-EM

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The spliceosome is a highly dynamic molecular machine whose composition is not static and whose structure undergoes several rearrangements during each cycle of splicing. A particularly dramatic structural rearrangement of the spliceosome occurs during the transition from the pre-catalytic B complex, which contains all of the spliceosomal snRNPs U1, U2, U4/U6 and U5, to the activated B^{act} complex. This remodeling step involves the dissociation of the U1 and U4 snRNPs, allowing the U6 snRNA to basepair with both the branchsite-bound U2 snRNA and the 5'end of the intron, forming a network of RNA-RNA base pair interactions that is thought to be at the heart of the spliceosome's catalytic center. While the biochemical composition of the various assembly stages of the yeast spliceosome is relatively well known, the ultrastructural study of isolated yeast spliceosomes is at a relatively early stage. Recently, we presented initial, high-quality electron microscopy images of yeast B and B^{act} spliceosomes (Mol Cell, 2009, 36:593). 2D images of the B^{act} complex exhibit a mushroom-like appearance, consisting of a main, slightly asymmetric body (the "mushroom cap"), from which a "mushroom stalk" is seen to emerge as a slightly tapered, ca. 15 nm-long protuberance.

A first step in understanding the spliceosome's architecture is to localize the position of individual spliceosomal components and functional centers. To topographically locate the emerging catalytic center of the purified yeast B^{act} complex, we first determined by DNA oligonucleotide-directed RNAse H digestion, accessible regions of the pre-mRNA closest to nucleotides of the 5' splice site and branch site. For EM localization studies, biotinylated 2'-OMe-RNA oligonucleotides were annealed to these accessible regions. The position of the bound oligonucleotides was then visualized by EM at the surface of the B^{act} complex using anti-biotin antibodies. We also inserted MS2 hairpin loops at defined positions of the exons and the intron, and mapped their positions in the B^{act} complex as well. The identified positions in the B^{act} EM map give first hints as to the path of the pre-mRNA in the yeast B^{act} complex and further define the possible location of the emerging catalytic core at the center of the mushroom cap.

Splicing Regulation

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 489 A – 528 A, 723 A
Location: Main Hallway & Sanada Foyer

489 A **Chromatin affects the splicing efficiency by regulating the U2snRNP activity**
492 A **PTB regulates the alternative splicing of the apoptotic gene BCL-X**
495 A **The Nuclear Matrix Protein Matrin 3 is a Regulator of Alternative Splicing**
498 A **Is Prp16 remodelling of helix I during the two steps of pre-mRNA splicing carried out through the Nineteen Complex protein Cwc2?**
501 A **Assessing influence of mutations in first nucleotides of exons on splicing of the BTK and SERPING1 genes.**
504 A **SR proteins regulate from cellular environment dependent splicing**
507 A **Thailanstatins: New Pre-mRNA Splicing Inhibitors and Potent Antiproliferative Agents Discovered from Burkholderia thailandensis MSMB43**
510 A **Sex-specific alternative splicing in the head of Drosophila melanogaster and its underlying regulatory mechanisms**
513 A **The centrosomal kinase NEK2 is a novel splicing factor kinase**
516 A **Functional characterization of the RNA-binding protein Acinus: its role in pre-mRNA processing and apoptosis**
519 A **Unusual evolutionary insertion of G-tracts creates splice variants of distinct localization and function in human cells**
522 A **Prp40p WW Domain is Critical for Splicing of Introns Containing Non-canonical Branch Site Sequences in Saccharomyces cerevisiae**
525 A **Splice-sensitive array profiling suggests a role for STAR proteins and PTB in control of smooth muscle cell alternative splicing.**
528 A **hnRNP A1 and Secondary Structure Coordinate Alternative Splicing of Mag**
723 A **Functional link between U1 snRNA 5'-end AU di-nucleotides and the mRNA cap-binding complex**

489-A Chromatin affects the splicing efficiency by regulating the U2snRNP activity

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A number of studies propose a role of the chromatin in the regulation of splicing. Many of these observations are based on *in vivo* experiments therefore a caveat of these studies is their correlative nature. Here, we present an *in vitro* system that reproduces the coupling of transcription/splicing, chromatin remodeling/transcription, and, for the first time, chromatin remodeling/splicing. Using this system, we demonstrate that the chromatin regulates the splicing efficiency in a co-transcriptional manner, independently of the recruitment of SR proteins by Pol II. Thus, we hypothesized that chromatin factors could have a structural role in the regulation of splicing. Using a high-throughput siRNA screen, we identified several chromatin factors affecting splicing, among which are regulators of acetylation and methylation of histones, chromatin remodelers, transcription regulators and nucleosome components. Using the U2snRNP to purify spliceosome, we show that several of these chromatin factors are physically associated to the splicing machinery, suggesting a functional coupling between the chromatin and the U2snRNP. Consistent with this proposal, alternative exons controlled by the active U2snRNP[1] are miss-regulated in absence of specific chromatin factors. These findings allowed us to propose a model in which chromatin modulates efficiency of splicing through the co-transcriptional regulation of the U2snRNP activity.

Reference:

[1] Nuclear Matrix Factor hnRNP U/SAF-A Exerts a Global Control of Alternative Splicing by Regulating U2 snRNP Maturation. Xiao R. et al., Mol. Cell., 2012

492-A PTB regulates the alternative splicing of the apoptotic gene BCL-X

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Alternative splicing is a key cellular mechanism to control gene expression, which is frequently altered in cancer cells. Mutations in splice sites, or regulatory sequences, as well as aberrant expression of splicing factors can contribute to defective splicing in cancer cells. Interestingly, changes in splicing regulation were shown to correlate with disease progression, whereas the specific splicing signature of cancer cells was employed to stratify patients with high accuracy.

An example of splicing-regulated gene with strong relevance for cancer is *BCL-X*. Alternative usage of the 5' splice sites located in exon 2 of *BCL-X* promotes the generation of two splice variants, the anti-apoptotic long variant (*BCL-XL*) and the pro-apoptotic short variant (*BCL-Xs*). Here, we identified the hnRNP I (PTB) protein as a novel regulator of *BCL-X* splicing. PTB was isolated by RNA chromatography as one of the splicing factors that associate with the alternatively spliced region of exon 2. Overexpression of PTB in HEK293T cells modulates 5' splice site selection in *BCL-X* exon 2, thereby favouring the pro-apoptotic *BCL-Xs* variant. Conversely, depletion of PTB promotes the BCL-XL variant. Crosslink immunoprecipitation (CLIP) experiments showed that PTB directly binds *BCL-X* exon 2 RNA in a sequence-dependent manner.

PTB is up-regulated in human glioblastoma and ovarian cancer. The PTB gene encodes at least three splice variants, named PTB1, PTB2 and PTB4. Interestingly, we observed that PTB1 was more efficient than PTB4 in promoting *BCL-Xs*. Moreover, by analysing several glioblastoma cell lines originated from tumours at different stages, we observed a positive correlation between the BCL-X S/L and the PTB1/PTB4 ratio. This finding suggests that a switch in PTB variants may favour the reduced expression of the pro-apoptotic form of BCL-X in glioblastoma cells. We are currently addressing this issue by evaluating apoptosis and cell survival in glioblastoma cells selectively silenced for PTB4 or for both variants.

Thus, our results identify *BCL-X* as a new splicing target for PTB and uncover a potential novel role for this RNA binding protein in the regulation of apoptosis.

495-A The Nuclear Matrix Protein Matrin 3 is a Regulator of Alternative Splicing

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Polypyrimidine Tract Binding protein (PTB) regulates an extensive range of alternative splicing events (ASE), and is composed of four RRM domains. In an MS2-tethering assay the second RRM and the following linker are sufficient to promote exon skipping. Short linear peptide motifs of the form [S/G][I/L]LGxfP, known as PTB RRM Interacting motifs (PRI), which are present in multiple copies in the PTB-coregulator Raver1, bind to the "dorsal" surface of RRM2. A key Tyr247 residue in RRM2 is critical for this interaction. Seeking to better understand the mechanism of splicing regulation by PTB, we used proteomics to identify proteins that bind to the PTB minimal repressor domain and are sensitive to mutation of Tyr247. The two strongest interactors were Raver1 and the nuclear matrix protein Matrin 3. The interaction with Matrin3 was mediated by a conserved GILGPPP motif, which is both necessary and sufficient for interaction with PTB. Matrin3 is composed of two DNA binding zinc-finger domains as well as two tandem RRMs. It is known to interact with RNA processing and transcription factors, and a mutation in this protein is associated with a type of distal myopathy, although its precise molecular function is unclear. We tested the consequences of Matrin3 knockdown in HeLa cells using splice-sensitive microarrays. Multiple ASEs were strongly affected, suggesting the activity of Matrin3 both as a splicing repressor and activator. There was a significant overlap between ASEs regulated by Matrin3 and PTB, but strikingly the majority of Matrin3 events were not co-regulated by PTB. Matrin3 targeted events showed a significant enrichment for chromatin proteins. Structure-function analyses indicated that the ZF domains are dispensable but Matrin3 requires its RRMs for splicing activity suggesting it binds directly to RNA. A number of 5-mer motifs are significantly enriched around Matrin3 repressed exons and adjacent to the downstream constitutive exon, including pyrimidine-rich motifs similar to optimal PTB sites. These motifs were also enriched among Matrin3 target exons that are not regulated by PTB. Strikingly, we found that Matrin3 activity was abolished by mutations of its GILGPPP motif for both PTB co-regulated and PTB-independent events, suggesting that this motif can mediate interactions with other splicing regulators. Our data indicate that Matrin 3 is not only functional as a nuclear matrix component but also as an active splicing regulator in the nucleoplasm.

498-A Is Prp16 remodelling of helix I during the two steps of pre-mRNA splicing carried out through the Nineteen Complex protein Cwc2?

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The DEAH box ATPase Prp16 has dual functions during the two catalytically steps of splicing. In the first catalytically step, in the absence of ATP, Prp16 stabilizes the binding of Cwc25 to the spliceosome and proofreads 5'splice site cleavage. To allow the second step of splicing Prp16, in an ATP-dependent manner, releases Cwc25 to allow for the binding of Prp22, Prp18 and Slu7. Because mutations in U2/U6 helix I are able to suppress a mutation in PRP16, it is thought that helix I is destabilized/remodelled by Prp16 between the two steps of splicing.

Cwc2 is a splicing factor that is part of the Prp19 complex (NTC). Cwc2 contains a Torus domain, an RNA recognition motif (RRM) and interacts directly with Prp19. Previously we showed that Cwc2 interacts directly with the U6 snRNA. We have now performed a large scale genetic analysis of Cwc2 mutants and over 80 snRNA mutants and found only one synthetic lethal interaction between Cwc2 and U6 in helix I. Mutations in the Cwc2 N-terminal Torus domain combined with the U6 mutation A56C, U57C in U2/U6 helix I leads to lethality because this U6 mutation destabilizes U2/U6 base-pairing. This lethality can however be suppressed by mutations that restore helix I base pairing between U2 and U6, suggesting that Cwc2 participates in U2/U6 helix I formation and/or stabilization.

We hypothesize that Cwc2 may be the target of Prp16 helix I destabilization/remodelling as Cwc2 directly interacts with helix I through the whole splicing cycle. Mutation in a conserved region of the Cwc2 Torus domain is able to suppress the cold sensitive *prp16-302* phenotype indicating a functional interaction between Cwc2 and Prp16. Initial crosslinking analysis suggests that Cwc2-U6 interaction is altered with a Prp16 first step mutant but is not altered with a dominant negative Prp16 second step mutant. As Prp16 does not directly interact with U2/U6 helix I, we propose that the destabilization/remodelling of U2/U6 helix I by Prp16 is carried out through Cwc2. We are currently investigating whether other spliceosomal ATPases exert their activity through the NTC.

501-A Assessing influence of mutations in first nucleotides of exons on splicing of the BTK and SERPING1 genes.

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Mutations in the first nucleotide of exons (E^{+1}) mostly affect splicing when found in AG-dependent 3' splice sites (3' ss), whereas exons with AG-independent splice sites are resistant to this type of mutation. In introns, AG-dependency is determined by the quality of the polypyrimidine tract, its length, and degeneracy. Because of this complex interplay between interdependent factors the mRNA splicing outcome of substitutions in 3' ss may be difficult to assess just from primary nucleotide sequence data. Recent study showed that mutations in the +1 G impaired splicing in exons with preceding polypyrimidine stretches (PPS) from 4 nt to 10 nt long whereas those with PPS from 9 to 16 nt long were normally spliced.[1]

In order to test this rule, we have used a minigene system to analyze the influence of five +1 G substitutions found in *BTK* and *SERPING1* genes on pre-mRNA splicing. Two of the mutations with the PPS length of 8 and 10 nts led to splicing aberration, while the other three with PPS length of 6; 6 and 8 nts did not affect the splicing. The two groups of sequences were slightly better distinguishable from each other according to the number of pyrimidines in 25 nts from the 3' ss, although we detected one outlier in the non-aberrant group.

Next, we examined how the most commonly used *in silico* splicing prediction tools would cope with the effect of these mutations on splicing. Both predictions of polypyrimidine tracts according to Kol and Schwartz failed in the discrimination between the AG-dependent and AG-independent 3' ss. Much better were the outcomes of splice site predictors. In the NNSPLICE program the border value discerning between the two groups of sequences was 7 % difference of the splice site score. One of the aberrant and one of the non-aberrant species showed that value. Analogous border value in the MaxEnt predictor was 20 % score difference, which clearly discerned the sequences according to their AG-dependency. Excellent results gave the PSSM predictions that showed 1 % score difference for all splicing non-affecting variants and 3 % difference for both splicing-affecting variants. We conclude that these *in silico* tools may be helpful for assessing the effect of a +1 G mutation on splicing. However, rather than generally recommended minimum of 10 % score difference for splicing affecting mutations, one should use values specific for each prediction tool.

1. Fu Y, Masuda A, Ito M, Shinmi J, Ohno K, Nucleic Acids Res 39 (2011) 4396-4404.

504-A SR proteins regulate from cellular environment dependent splicing

Egle Jakubauskiene[1], Egle Jakubauskiene[1], Laurynas Vilys[1], Yuichi Makino[3], Lorenz Poellinger[2], Arvydas Kanopka[1]

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Oxygen (O_2) is essential for the life of all aerobic organisms. In mammals, at the whole body level, oxygen supply is optimized by tight regulation of ventilation, arterial blood hemoglobin saturation and systemic oxygen transport. The oxygen tension has been demonstrated to be a key regulator to optimize specific organ functions.Hypoxia-inducible factors (HIF-1a; HIF-2a; HIF-3a) are a transcriptional complex that plays a central role in oxygen-regulated gene expression. HIFs DNA binding complex consists of a heterodimer of HIF-a and HIF-ß - identical to the previously identified arylhydrocarbon receptor (AhR) nuclear translocator (ARNT). All these proteins belong to the family of basic helix-loop-helix (bHLH) proteins that contain a PAS domain. HIFs bind to hypoxia responsive elements (HRE) and activate the transcription of a variety of genes involved in the regulation of erythropoiesis, angiogenesis, vasomotor control and energy metabolism.

In mice a dominant negative regulator of hypoxia-inducible gene expression (IPAS) is generated in hypoxic cells by alternative splicing from HIF-3a pre-mRNA. In this study, we aim to understand the molecular mechanisms, regulation of which underlay the alternative HIF-3a/IPAS pre-mRNA splicing process.

We report that: 1) HIF-3a splice sites are efficiently used in HeLa cell nuclear extracts prepared under normoxic conditions, and very inefficiently in HeLa cell nuclear extracts prepared under hypoxic conditions; 2) IPAS splice sites used very inefficiently in HeLa extracts prepared under normoxic conditions, and used in HeLa cell nuclear extracts prepared under hypoxic conditions; 3) UV crosslinking results revealed that SR proteins isolated from normoxic and hypoxic cells interact with RNA differentially.Regulation of HIF-3a/IPAS pre-mRNA splicing dependent from oxygen tension is under further investigation.

507-A Thailanstatins: New Pre-mRNA Splicing Inhibitors and Potent Antiproliferative Agents Discovered from Burkholderia thailandensis MSMB43

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More than 90% of human genes undergo alternative slicing, which results in protein variants far greater than the number of the encoding genes. While this processing inherently provides for transcriptome diversity, aberrant alternative splicing has been implicated in numerous diseases conditions such as cancer and neurodegeneration. The cellular machinery of alternative splicing has thus become a valid drug target, and dozens of small molecule effectors interrogating the alternative splicing process have been identified and evaluated as drug candidates. The aim of our research is to discover new natural products from rare bacterial species that target eukaryotic epigenetics and gene transcriptional regulation including alternative splicing. Mining the genome sequence of *Burkholderia thailandensis* MSMB43 revealed a cryptic biosynthetic gene cluster highly resembling that of FR901464, a prototype pre-mRNA splicing inhibitor produced by *Pseudomonas* sp. No. 2663. Transcriptioal analysis identified a cultivation condition in which a key gene of the cryptic gene cluster is adequately expressed. Consequently, three new compounds, named thailanstatins A, B and C, have been isolated from the fermentation broth of *B. thailandensis* MSMB43 through natural product chemistry (see Figure 1 below). Thailanstatins belong to the FR901464-family of microbial products biosynthesized by a hybrid polyketide synthease-nonribosomal peptide synthetase pathway. They have an overall structural similarity with FR901464, but differ by lacking an unstable hydroxyl group and by having a carboxyl moiety which together endow the compouds with a significantly greater stability than FR901464 under physiologically relevant conditions. *In vitro* assays showed that thailanstatins inhibit pre-mRNA splicing as potently as FR901464, with half-maximal inhibitory concentrations in the single to sub mM range, causing pre-mRNA to accumulate and preventing the production of mRNA and splicing intermediates. *In vitro* cell culture assays indicated that thailanstatins also possess potent antiproliferative activities in representative human cancer cell lines, with half-maximal growth inhibitory concentrations in the single nM range (see Table 1 below). This work provides new chemical entities as reagents for research and as drug candidates for development, and validates the *Burkholderia* species as an exciting new source of bioactive natural products.

Figure 1. Chemical Structure of Potent Pre-mRNA Splicing Inhibitors

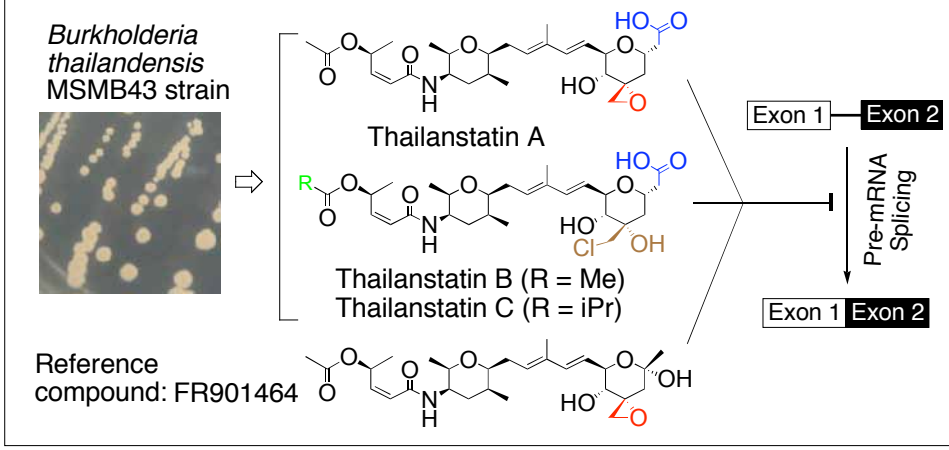

Table 1. Properties of Thailanstatins in comparison with FR901464

Natural Product	Half-life ($t_{1/2}$, hr)	Pre-mRNA splicing inhibitory activity (IC_{50} in μM)	Antiproliferative activity (GI_{50} in nM)			
			DU-145 (prostate cancer cell line)	NCI-H232A (non-small cell lung cancer cell line)	MDA-MB-231 (triple-negative breast cancer cell line)	SKOV-3 (ovarian cancer cell line)
Thailanstatin A	>78	0.65±0.36	1.11±0.02	2.26±0.17	2.58±0.11	2.69±0.37
Thailanstatin B	19	6.18±2.47	3.00±0.92	2.50±0.06	6.22±1.67	4.94±1.76
Thailanstatin C	25	6.84±2.90	2.98±0.90	3.67±0.53	8.82±2.20	5.57±2.01
FR901464	10	0.58±0.07	1.05±0.02	1.94±0.24	2.10±0.19	1.06±0.01

510-A Sex-specific alternative splicing in the head of Drosophila melanogaster and its underlying regulatory mechanisms

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Drosophila sex-determination is a prime example of an alternative splicing cascade where Sex-lethal (SXL) as a master regulator controls alternative splicing of the key sex-determination genes *Sxl*, *transformer (tra)*, *male-specific-lethal* (*mls-2)*, *doublesex (dsx)* and *fruitless (fru)*.

The recent discovery that the number of sex-specifically spliced genes is magnitudes higher than expected, suggests a more extensive post-transcriptional regulation program, which may act together with transcriptional changes to shape sex determination in *Drosophila melanogaster*. The question arises of how these events are regulated. Interestingly, some of the sex-specific alternative splicing (AS) changes in adult fly heads are lost in genetically manipulated flies lacking a germline, suggesting that signals from the germline control sex-specific AS in distant tissues. A similar scenario might be true for male-derived accessory gland proteins that are transferred via the seminal fluid during copulation from males to females. We use the *Drosophila* model system to identify new regulatory concepts in sex-determination and take advantage of the genetic accessibility of *Drosophila* to study alternative splicing directly *in vivo*.

To determine to what extent sex-specific AS is present in the head, and what influence the germline and mating process have on the AS pattern, we analyzed the transcriptome of adult wild type, tud, and virgin female and male fly heads, using Next Generation Sequencing (NGS). Significant sex-specific expression changes were observed in 218 genes and AS changes in 230 genes (corresponding to 407 AS events). In addition, the experiments showed that AS is highly regulated in the heads of germline-less and virgin flies. Indeed, first analysis of the data suggests that appr. 30-40 % of the AS events in the head depend on the germline and mating process, respectively.

Dual fluorescence minigene reporters are excellent tools to monitor AS, but have not been used in *Drosophila* so far. As tools for cell culture and *in vivo* experiments, these reporters can be used to identify trans-acting and cis-acting regulatory elements of the splicing process. For large scale analysis, AS of these reporters in cell culture can be analyzed using FACS. For different promising candidate genes of the NGS experiments, AS reporters are being established and the data will be presented. For example, we constructed a dual fluorescence reporter minigene to monitor s*qd* AS, using its endogenous promoter. Differences in AS of *sqd* were recapitulated in *Drosophila* S2 cells. Differential isoform expression, using transgenic flies carrying this reporter was detected in the cortex of the nervous system with one isoform showing higher levels in the mushroom body.

This established system enables to monitor AS directly *in vivo* and allows to identify trans-acting and cis-acting regulatory elements of the splicing process.

513-A The centrosomal kinase NEK2 is a novel splicing factor kinase

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NEK2 is a serine/threonine kinase belonging to the family of the NIMA kinases, which are well known as key regulators of the cell cycle. In particular, NEK2 promotes centrosomes splitting and insures correct chromosomes segregation during the G2/M phase of the cell cycle through the phosphorylation of specific substrates, such as the centrosomal protein c-Nap1. Aberrant expression and activity of NEK2 leads to dysregulation of the centrosome cycle and aneuploidy. Thus, a tight regulation of NEK2 activity and expression is needed during cell cycle progression.

NEK2, as other centrosomal kinases, is up-regulated in several human cancers, such as breast carcinoma, testicular seminoma, and myeloma, and its expression levels have been proposed as an accurate prognostic marker.

In both testicular seminomas and myelomas, NEK2 overexpression correlates with its nuclear localization. This observation suggests the existence of unknown nuclear functions for NEK2 in cancer cells, which have been object of our further investigation.

We found that NEK2 localizes in the nucleus of cancer cells derived from several different tissue and in particular it localizes in their splicing speckles. Moreover, NEK2 interacts with several splicing factors and phosphorylates some of them, like the proto-oncogene SRSF1. Overexpression of NEK2 exerts the same effect of the SR protein kinase SRPK1 on phosphorylation of endogenous SR-proteins. Moreover, NEK2 and SRPK1 similarly affect the splicing activity of SRSF1 towards reporter minigenes and its endogenous targets.

Our results identify NEK2 as a novel kinase involved in splicing regulation, suggesting that part of its oncogenic activity may be ascribed to its ability to modulate this key step in the regulation of gene expression, which is frequently altered in cancer cells.

516-A Functional characterization of the RNA-binding protein Acinus: its role in pre-mRNA processing and apoptosis

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Acinus (Apoptotic Chromatin Condensation Inducer in the Nucleus) is an RNA-binding protein originally identified for its role in inducing nuclear changes during apoptosis (1). This protein has also been found to be an auxiliary component of the Exon Junction Complex (EJC), which is deposited at exon junctions as a consequence of pre-mRNA splicing (2). A role for Acinus in splicing is also suggested by its association with RNPS1 and SAP18 proteins in the ASAP (apoptosis- and splicing-associated protein) complex.

In order to uncover the cellular functions of Acinus, we searched for endogenous RNA targets using the Cross-Linking Immunoprecipitation protocol (iCLIP) that allows the mapping of protein-RNA interactions at an individual nucleotide resolution. We found that Acinus is mostly associated with constitutively expressed exons of protein-coding transcripts. Interestingly, Acinus binding is excluded from the region where the core EJC is deposited (20/24nt before the exon-exon junction). We also identified non-coding RNAs targets of Acinus.

An exon-junction array was used to investigate changes in gene expression and alternative splicing following siRNA-mediated depletion of Acinus in HeLa cells. This analysis revealed changes in expression levels of around 450 genes as well as changes in 250 splicing events. A large number of genes presenting a change in alternative splicing were associated with an Acinus binding site, suggesting a direct role of this RNA-binding protein in the event. We are also investigating whether the role of Acinus during apoptosis involves its binding to specific RNAs. The combination of these approaches will help us to uncover the role of Acinus in pre-mRNA splicing, apoptosis and other cellular processes.

1. Sahara et al. (1999) Nature, 401, 168-73.
2. Tange et al. (2005) RNA, 11, 1869-83.

519-A Unusual evolutionary insertion of G-tracts creates splice variants of distinct localization and function in human cells

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Alternative splicing contributes greatly to the proteomic complexity in higher eukaryotes particularly humans. However, the underlying molecular mechanisms for the evolvement of alternative exons during evolution and its functional consequences remain largely unknown. The polypyrimidine tract (Py) and 3' AG are often close to each other within a consensus sequence (Y)nNYAG at the 3' splice site. In contrast to this arrangement for exon inclusion, here we report an unusually evolved G-tract insertion between the Py and AG for exon skipping, and consequently a variant protein of distinct localization and function in human cells.

We identified 130 3' splice sites containing G-tracts between the Py and 3' AG in a human genome search. Interestingly, examination of several such elements indicates that they are inserted evolutionarily in higher species. Particularly G-tracts upstream of the exon 3 of the PRMT5 (protein arginine methyl transferase 5) gene are gradually evolved from none in fish to one in certain lower and two in higher mammals. Contrary to the intronic G-tracts at other locations that are often splicing enhancers, these G-tracts are splicing repressors in mini-gene splicing reporter assays. Moreover, the repression is strong in humans but barely detectable in fish for both mini-gene reporter and endogeneous exons. In *in vitro* UV crosslinking and immunoprecipitation assays, the G-tract element is bound by hnRNP H and inhibitory of U2AF65 binding to Py. Consistently, hnRNP F/H knockdown enhances the inclusion of endogenous exon 3 in HeLa cells. Together these suggest that the unusually localized G tracts are evolutionarily inserted splicing repressors bound by a regulatory protein in human cells.

To understand the consequences of the G-tract-mediated skipping of exon 3 in humans, we examined the expression profiles and expressed each of the splice variant separately in cells. The variants are differentially expressed among cells or during differentiation. With exon 3 included, a full length protein is produced but restricted to discrete Golgi-like structures while as with the exon skipped, a shorter protein is produced and spread all over the cell. Consistent result is obtained when each variant was knocked down by siRNA interference. Importantly, the full length but not the shorter isoform greatly increases the fragmentation of the Golgi apparatus in co-immunostaining assays with an antibody against the Golgi marker Giantin. Therefore, the two protein isoforms have distinct localization and function.

These data demonstrate that the unusual evolutionary insertion of G-tracts creates an alternative exon of distinct localization and function in human cells. This is likely a mechanism common to the emergence of a group of alternative exons that contribute to the proteomic complexity in humans. *Supported by The Natural Sciences and Engineering Research Council of Canada (NSERC).*

522-A Prp40p WW Domain is Critical for Splicing of Introns Containing Non-canonical Branch Site Sequences in Saccharomyces cerevisiae

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The WW domain is a protein domain with two highly conserved tryptophans that binds proline-rich peptide motifs. Prp40p is a yeast-specific and essential U1-snRNP protein that harbors two WW repeats at its N-terminus, which were thought to interact with the proline-rich region of BBP (**b**ranch-site **b**inding **p**rotein) for promoting the formation of commitment complexes. Surprisingly, earlier studies by others showed independence of the conserved WW repeats in splicing and contranscriptional spliceosome assembly. We have carefully approached the same issue and discovered that WW repeats are in fact critical for splicing of introns containing non-canonical branch site sequences (e.g. GAUUAAC vs. canonical UACUAAC) in vivo. To exclude the possibility of gene-specific bias, we employed a splicing reporter system and found that when the branch-site sequence is changed into GAUUAAC, splicing became sensitive to the loss of either WW repeats and, most apparently in particular, the loss of both WW repeats. This sensitivity appears most dramatic when cell growth was assayed at 37°C. To further determine in detail the WW repeats' function, and therefore Prp40p's, in splicing, we used splicing-sensitive microarray to identify intron-containing genes that are susceptible to the loss of Prp40p WW repeats. To enhance our understanding of the function of the Prp40p-WW repeats from an entirely different angle, we searched for gene deletions that cause synthetic lethality or sickness in combination with the *prp40-?N* mutation in the Synthetic Genetic Array (SGA) platform. Finally, we applied the BPA chemical cross-linking approach to directly test the hypothesis that the WW repeats indeed interact with the proline-rich region in BBP in vivo. In summary, we uncovered a hitherto hidden splicing role for the WW domain of Prp40p in the light of their relationships to the role of BBP. Because the branch site sequences is less conserved in mammals than that in yeast, the WW-domain containing proteins in mammals may play regulating roles at the branch site recognition in splicing.

525-A Splice-sensitive array profiling suggests a role for STAR proteins and PTB in control of smooth muscle cell alternative splicing.

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Tissue-specific alternative splicing has been extensively investigated in striated muscles (heart and skeletal muscle), and a large amount is known about the relevant RNA sequence elements and RNA binding proteins involved. By contrast, the regulation of alternative splicing in smooth muscle cells (SMCs) has been relatively neglected. Vascular SMCs show phenotypic plasticity and can interconvert between a differentiated 'contractile' phenotype and a more proliferative 'synthetic' phenotype marked by increased synthesis of extracellular matrix proteins. This phenotypic modulation process plays a significant pathophysiological role in various cardiovascular diseases. While the transcriptional changes during phenotypic modulation have been well investigated, knowledge about the global changes in alternative splicing has been very limited.

We have used splice-sensitive microarrays to interrogate global changes in both transcript levels and alternative splicing during phenotypic modulation of mouse aorta and bladder smooth muscle. Genes affected by alternative splicing showed distinct functional enrichments from those that were transcriptionally up or down-regulated. Splicing particularly affected cytoskeletal proteins, while ion channels and receptors were transcriptionally down-regulated and receptor binding and extracellular matrix proteins were upregulated. Nucleic acid binding proteins were significantly depleted among the transcriptionally regulated genes. We identified sets of cassette exons that were substantially up or down-regulated during phenotypic modulation. Computational analysis showed that exons that are included in contractile SMCs are associated with PTB-binding motifs on the upstream side, where PTB represses splicing. Downstream of these exons there was a substantial enrichment of motifs resembling the binding sites for members of the signal transduction and activation of RNA (STAR) protein family (UUAAC, UAACC, ACUAA, CUAAC), which have not previously been associated with regulation of splicing in SMCs. Candidate STAR-regulated exons with potential binding sites in downstream intron were manually identified from 50 top-ranked events and the splicing pattern changes of 7 events (*Ncam1* exon 2, *Atp2b4* exon 20, *Cacna2d1* exon 23, *Ppp4r1* exon 3, *Sfrs10* exon 2, and *Bnip2* exon 10, *Ppp1r12a* exon 24) were validated by RT-PCR. In preliminary experiments using an *Ncam1* exon 2 minigene reporter in proliferative rat PAC1 cells, overexpression of Sam68, SLM1 and SF1 significantly increased *Ncam1* exon 2 inclusion, with Sam68 being the strongest regulator. These initial observations suggest that STAR proteins might act widely to promote inclusion of cassette exons in contractile SMCs, while many of the same exons might be repressed by PTB in de-differentiated cells.

528-A hnRNP A1 and Secondary Structure Coordinate Alternative Splicing of Mag

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Myelin is a lipid-rich structure that protects neurons from degeneration and facilitates the propagation of electrical impulses along axons. In the central nervous system, it is formed by a specialized glial cell called an oligodendrocyte, which extends hundreds of spiraling processes to nearby axons and ensheathes them. A major protein component of myelin in the vertebrate central nervous system is myelin-associated glycoprotein (MAG). MAG is positioned at the periaxonal interface of the myelin structure, where its position allows it to mediate communication between the neuron and the oligodendrocyte, inhibiting axon outgrowth and protecting neurons from degeneration. The *Mag* pre-mRNA is alternatively spliced to produce two developmentally regulated transcripts. The longer mRNA contains an alternative cassette exon that has a stop codon, yielding a protein with a truncated C-terminus. How *Mag* alternative splicing is regulated is not clear. Here, we describe an evolutionarily conserved stem loop structure that overlaps the *Mag* exon 12 5' splice site. The non-consensus 5' splice site occupies the loop of the stem and is able to interact with the splicing repressor hnRNP A1. Analysis of a series of splicing reporters shows that both the sequence and the structure of the element regulate *Mag* alternative splicing.

723-A Functional link between U1 snRNA 5'-end AU di-nucleotides and the mRNA cap-binding complex

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Binding of the U1 snRNP to pre-mRNA 5' splice site (5'ss) plays a critical role in splicing by committing the pre-mRNA substrate to the splicing pathway. The interaction between U1 snRNP and the 5'ss is mediated in part by Watson-and-Crick basepairing of the U1 snRNA's 5'-end (from the 3rd positions) to the relatively conserved 5'ss (GUAUGU, in the budding yeast). Intriguingly, despite no apparent participation of the first two AU residues of the U1 snRNA in interacting with the 5'ss, these two positions are highly conserved from fungi to metazoans. To investigate the role of these two residues in splicing, we systematically mutated them, at the U1 snRNA gene (*SNR19*) level, to all possible combinations. The majority of mutants exhibit no statistically significant difference in terms of fitness from that of the wild-type cells, suggesting that the AU dinucleotides may not be crucial. However, the AUto-UU (AU>UU) mutant is greatly compromised in fitness and exhibits a cold-sensitive phenotype. Primer-extension analysis shows that the otherwise unique transcriptional start of *SNR19* in the AU>UU mutant is dramatically altered and results in multiple 5'-end-truncated and non-functional U1 snRNAs. Quantitative real-time PCR as well as **splicing-sensitive microarray** analysis show a global accumulation of pre-mRNAs, **suggesting that** insufficient production of functional U1 snRNA in the AU>UU mutant significantly compromises splicing. Moreover, genome-wide synthetic-lethal screen uncovered two categories of genes that are functionally related to the AU>UU mutation: splicing as well as transcription-related genes. Detailed analysis pinpoints that the most appealing candidates are the normally nonessential cap-binding proteins, Cbp20p and Cbp80p, which become indispensible upon altering the AU di-nucleotides in essentially all variations. This result thus strongly suggests that the AU di-nucleotides indeed have a role in splicing mediated through the cap-binding complex. The mechanistic details of this novel link are being defined biochemically.

RNA-Protein Interactions

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 531 A – 579 A
Location: Main Hallway & Sanada Foyer

531 A **Defining a eukaryotic core mRNA interactome: the landscape of RNA-binding proteins in yeast and its conservation in mammals**
534 A **GOLLD: a large, structured, noncoding RNA from bacteria and bacteriophages**
537 A **The QUA2 domain of GLD-1 recognizes an additional nucleotide and modulates RNA binding affinity**
540 A **Interactions between RBFOX2 and pre-microRNA-20b terminal loop**
543 A **Potential substrates of the RNase MRP complex in cell cycle regulation**
546 A **A novel PAR-CLIP based approach using RRM mutations reveals RNA recognition mechanisms of HuR**
549 A **Testing Protein Sequestration Candidates for RNA-Mediated Disease**
552 A **Establishment of a Fluorescence Cross Correlation Spectroscopy (FCCS) based assay to measure Argonaute – siRNA interaction**
555 A **The RNA-binding Protein Repertoire of Embryonic Stem Cells**
558 A **Multifunctional interleukin-6 receptor aptamers**
561 A **Knock-out mice and HITS-CLIP reveal that the SGs assembly factor G3BP preferentially binds intron-retaining transcripts and controls their stability in the brain**
564 A **Sequence specific code in RRM–RNA interactions**
567 A **Manipulating endogenous RNAs with synthetic RNA-binding proteins**
570 A **Architecture of catalytic complexes essential for synthesis and co-translational insertion of selenocysteine in humans**
573 A **HIV Nucleocapsid Protein Precursors are Effective Nucleic Acid Chaperone Proteins**
576 A **Crystal structure of the active conformation of the Shigella flexneri VapC toxin at 1.9 Å resolution**
579 A **Responses of the mRNA interactome to genotoxic stress**

531-A Defining a eukaryotic core mRNA interactome: the landscape of RNA-binding proteins in yeast and its conservation in mammals

Benedikt Beckmann[1], Alfredo Castello[1], Bernd Fischer[1], Rastislav Horos[1], Claudia Strein[1], Katrin Eichelbaum[1], Sophia Föhr[1], Thomas Preiss[2], Lars Steinmetz[1], Jeroen Krijgsveld[1], Matthias Hentze[1]

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We recently defined the mRNA interactome of proliferating human HeLa cells, identifying 860 mRNA-binding proteins (mRBPs) and implicating more than 300 previously unknown RBPs in RNA biology [1]. We here report the next step forward, work on the unicellular yeast *Saccharomyces cerevisiae*. Yeast is of particular interest for its availability of advanced genetic tools for studies in vivo, and -in conjunction with the mammalian interactomes- allows definition of conserved RBPs and their function across evolution.

We developed an adapted protocol for interactome capture [2] in yeast based on PAR-crosslinking (Photoactivatable-Ribonucleoside-Enhanced Crosslinking). Using quantitative proteomics, we identified 678 high confidence (m)RBPs (FDR<0.01), including many previously unknown ones. We confirm 101 out of the 120 recently reported RBPs [3], and newly identify 283 additional high confidence RBPs.

Cross referencing with human mRNA interactomes [1, 4] defines 259 members of an evolutionarily conserved "core" mRNA interactome including many RBPs involved in central processes of RNA metabolism (e.g. processing, transport, translation). One of the most unexpected aspects is the occurence of several members of the oxidoreductase family that was previously unlinked to RNA biology. Among others, these include GAPDH, thioredoxin 1 and 2, and alcohol dehydrogenase 1 (ADH1). Moreover, the emerging core interactome includes a defined set of enzymes from diverse pathways in intermediary metabolism, suggesting connections between cell metabolism and the regulation of gene expression [5].

[1] Castello A, Fischer B et al. *Cell*. 2012; **149**(6)

[2] Castello A et al. *Nature Protocols*. 2013; **8**(3)

[3] Mitchell SF et al. *Nat Struct Mol Biol*. 2013; **20**(1)

[4] Baltz AG et al. *Mol Cell*. 2012; **46**(5)

[5] Hentze MW and Preiss T, *Trends Biochem Sci*. 2010; **35**(8)

534-A GOLLD: a large, structured, noncoding RNA from bacteria and bacteriophages

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The Giant, Ornate, Lake- and Lactobacillales-Derived (GOLLD) RNAs belong to a class of large, structured non-coding RNAs found in many bacteria and phages. The function of these RNAs is unknown, but their size and conserved structure are reminiscent of large, sophisticated ribozymes such as ribosomes, self-splicing introns, and RNase P[1]. More than 600 of these RNAs have been found in both bacteria and phages, and they are frequently encoded next to tRNA genes. Although little is known about GOLLD RNAs, they are a candidate for a new class of ribozyme.

Commonly, large ribozymes perform their functions in complex with other proteins or RNAs. Because GOLLD is a ribozyme candidate, we sought to identify any interacting partners, which may help elucidate the RNA function. Using formaldehyde-crosslinking, we developed a bead-assisted pulldown procedure to identify interacting molecules. Preliminary data shows that much more rRNA was pulled down when GOLLD was expressed, compared with when GOLLD was not expressed. Therefore, most of the rRNA in the bead-bound fraction appears to have been cross-linked to GOLLD. In addition, reverse-phase chromatography shows that GOLLD RNAs co-elute with the 23S ribosomal RNA, which is consistent with previous results, suggesting that GOLLD RNAs may be performing functions related to ribosomes. However, more in-depth experiments are required to rule out any spurious interactions.

1. Weinberg Z, Perreault J, Meyer MM, Breaker RR. Exceptional structured noncoding RNAs revealed by bacterial metagenome analysis. 2009. *Nature*. 462: 656-9.

537-A The QUA2 domain of GLD-1 recognizes an additional nucleotide and modulates RNA binding affinity

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The members of the STAR (signal transduction and activation of RNA) protein family are key players in post-transcriptional processing and are especially essential for developmental processes, including the germline and the vertebrate nervous system. Recent data hint to implications of these proteins in cancer [1] and neurological diseases like human inherited ataxia, multiple sclerosis or schizophrenia [2]. In order to gain more insights into the role of the STAR protein family in these diseases, it is inevitable to understand their molecular basis of RNA interaction.

All STAR proteins share a very high sequence similarity in their RNA-binding domain, an extended KH domain (KH-QUA2), and contain an N-terminal dimerization domain (QUA1), with the exception of SF1 [3] .The family member GLD-1 (Germline defective 1) is a germline specific translational repressor in *C. elegans* and was suggested to associate with 10% of all germline detected mRNA to date [4]. It is essential for germ cell development [5] and can therefore be seen as its key regulatory factor. GLD-1 (*Germline defective 1*) binds as dimer to a single, highly conserved TGE-repeat found within the 3' UTR of its mRNA targets. The importance of the RNA-binding domain for the function of GLD-1 is emphasized by critical mutations within the KH domain, leading to tumor formation or masculinization of the hermaphrodite germline [6]. While the mammalian homolog SF1 recognizes specifically a hexanucleotide RNA sequence, a recently conducted immunoprecipitation assay for GLD-1 proposed a consensus sequence spanning a heptanucleotide [7]. This emphasizes the need of structural data for the RNA binding domain of GLD-1, since the solution structure of SF1 is not sufficient to explain this difference in RNA recognition [8].

We determined the solution structure of the monomeric GLD-1 KH-QUA2 domain bound to its primary target site within the TGE repeat of the *tra-2* gene [9]. In contrast to SF1, the QUA2 domain adopts a slightly different orientation and is thus indeed able to specifically recognize an additional nucleotide with an increase in RNA-binding affinity. In addition, this structure provides a rational for previously published mutagenesis data and subsequently answers the remaining questions about RNA specificity of GLD-1. In conclusion, our data explains the differences to the homologous structure of SF1, provides thus a rational for previous studies on GLD-1 and forms an important basis to understand the impact of the QUA2 domain on RNA binding for the whole STAR protein family.

[1] Novikov et al, Mol Cell Biol, 2011
[2] McInnes & Lauriat, Neuroscience and biobehavioral reviews, 2006
[3] Artzt & Wu, Adv Exp Med Biol, 2010
[4] Scheckel et al, PLoS Genet, 2012
[5] Francis et al, Genetics, 1995
[6] Jones et al, Dev Biol, 1996
[7] Wright et al, EMBO J, 2011
[8] Liu et al, Science, 2001
[9] Jan et al, EMBO J, 1999

540-A Interactions between RBFOX2 and pre-microRNA-20b terminal loop

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Several RNA binding proteins originally known for their role in pre-mRNA processing have recently been found to also operate during the biogenesis of microRNAs. RBFOX2, a known regulator of alternative splicing, specifically binds to GCAUG motifs in the pre-mRNAs to regulate inclusion or exclusion of flanking exons. We identified several chemically synthesized pre-miRNA hairpins that bound to RBFOX2 *in vitro*. One of these, hsa-miR-20b contained the characteristic GCAUG recognition motif in the terminal loop region. As determined by surface plasmon resonance (SPR), the recombinant RRM domain of RBFOX interacted with the syn-pre-miR-20b with moderate affinity (3.6 μM). Mutation of G5 residue, which is essential for RBFOX2 binding to GCAUA completely abrogated the binding. The overexpression of miR-20b by transfection of its chemically synthesized precursor or by a pri-miRNA plasmid suppressed RBFOX2 protein levels. Upon G to A mutation in the loop, RBFOX2 suppression activity was lost. These data reveal a novel connection of RBFOX2 to microRNAs, in addition to alternative splicing.

543-A Potential substrates of the RNase MRP complex in cell cycle regulation

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The RNase MRP complex is composed of the RNA component of mitochondrial RNA processing endoribonuclease (*RMRP*) and five additional protein components. This RNAse MRP complex plays a role in the tailoring of the 5'end of the 5.8S rRNA.

RMRP is the first non-coding RNA which is associated with a human disease. Patients with cartilage-hair hypoplasia (CHH) have recessive mutations in *RMRP*; they are affected by skeletal dysplasia and a predisposition for various tumours. Recent studies have shown that *RMRP* also forms a complex with TERT (telomerase reverse transcriptase). This RNA-protein complex may have a RNA-dependent RNA polymerase activity. An isolated deficieny in TERT causes Diskeratosis congenital (DC). Intruigingly, the clinical manifestations of DC are very similar to CHH. In RNA-protein complex formation, the limiting factor is TERT. One consequence of impaired TERT function is progressive telomere shortening in proliferating cells. This results in cell cycle arrest and the induction of DNA repair mechanisms. Cell cycle genes such as p53 and p21 (also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1) play an important role in these processes. Malfunction of these genes may lead to tumor formation and cellular senescence.

A negative correlation between expression of TERT and *RMRP* level has already been shown in some cell lines. The interaction of *RMRP*, TERT, and cell cycle genes could provide an explanation for the tumour susceptibility of CHH patients.

We will present preliminary data on the role of *RMRP* in cell cycle regulation and senescence.

546-A A novel PAR-CLIP based approach using RRM mutations reveals RNA recognition mechanisms of HuR

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HuR is a ubiquitous, multi-functional RBP that contains three RNA recognition motifs (RRMs) which allows it to recognize several closely related low complexity U-rich elements including the AU-rich element (ARE). However, it is unclear how each of the RRMs contributes to recognizing these elements and whether they have distinct preferences among the global set of targets. This is especially true for the third RRM which is known to bind U-rich RNA but has also been suggested to bind to poly(A) sequences; moreover, RRM3 is thought to be pivotal for *in vitro* multimerization of HuR. To address this we have combined a mutational approach with a modified version of PAR-CLIP, a technique for identifying global RNA-protein interactions. Our novel approach includes correcting for previously unaccounted for background binding and using a combination of deletion and point mutants designed from published RRM/RNA co-crystal structures. Our data agrees with previous findings that all three RRMs are not required for RNA recognition but that at least two RRMs are required for binding to most sites. Furthermore, our data also demonstrate that RRM3 can recognize sequences similar to RRMs 1 and 2 and that inactivation of RRM3 doesn't dramatically alter the sequence preference of HuR. Interestingly, while the three RRMs are at least partially redundant for recognizing similar sequences our data suggests that they have unique roles in determining where HuR binds globally. Specifically, inactivation of RRM3 resulted in fewer HuR binding sites in close proximity to other HuR binding sites, especially within intronic sequences. This suggests that RRM3 initiates and/or stabilizes multimerization of HuR *in vivo*, a mechanism previously proposed to explain how HuR competes with miRNAs. However, since our data indicate multimerization is predominately in introns this may relate to the ability of HuR to regulate splicing as well. This mechanism of RRM3-dependent multimerization potentially extends beyond HuR and may apply to the roughly 100 other 3 RRM-containing RBPs, nearly all of which share similar inter-domain spacing patterns.

549-A Testing Protein Sequestration Candidates for RNA-Mediated Disease

552-A Establishment of a Fluorescence Cross Correlation Spectroscopy (FCCS) based assay to measure Argonaute – siRNA interaction

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Argonaute Proteins are the key players in the RNA interference mechanism (RNAi) by which double stranded RNA leads to the loss of an mRNA homologous sequence. Synthetic short interfering RNA duplexes (siRNAs) effectively can be used to downregulate certain genes of interest at the mRNA level. Therefore siRNAs targeting the same target RNA can exhibit different knockdown efficiencies or lead to unwanted off-target effects.

Here we report the measurement of the molecular interactions between siRNAs and Ago proteins via Fluorescence Cross Correlation Spectroscopy (FCCS). With this method information about the size, the rate of diffusion and concentration of each fluorescently labeled particle can be obtained. This is achieved by analyzing the time-dependent fluctuation of the fluorescence signal of Cyanine-5 (Cy5) labeled siRNAs and GFP fused Ago proteins through a confocal detection volume.

The aim of the project is to establish an FCCS based assay to measure direct interactions of Ago Proteins with siRNAs and to examine whether the affinity of a certain siRNA correlates with its knockdown potency *in vivo*. Furthermore this assay provides the possibility to identify potential regulatory sites of Argonaute proteins by comparing the binding kinetics of Ago mutants with the respective wildtype protein.

555-A The RNA-binding Protein Repertoire of Embryonic Stem Cells

S. Chul Kwon[1], Hyerim Yi[1], Katrin Eichelbaum[2], Sophia Föhr[2], Bernd Fischer[2], Kwon Tae You[1], Tuan Anh Nguyen[1], Alfredo Castello[2], Jeroen Krijgsveld[2], Matthias W. Hentze[3], V. Narry Kim[1]

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RNA-binding proteins (RBPs) play essential roles in RNA-mediated gene regulation, and yet the current annotation of RBP is largely limited to those with known RNA-binding domains. To systematically identify the RBPs of embryonic stem cells (ESCs), we here employ "interactome capture", which combines UV-crosslinking of RBPs to RNA in living cells, oligo(dT) capture, and mass spectrometry. From mouse ESCs, 555 proteins are defined here to constitute the mESC RNA-interactome, which includes 283 proteins not previously known to bind RNA. Interestingly, 39 novel RBP candidates are highly expressed in ESCs when compared to differentiated cells, suggesting that they may play important roles in stem cell physiology. Among them, two well-known E3 ubiquitin ligases (Trim25/Efp and Trim71/Lin41) are validated as novel RBPs, revealing a potential link between RNA biology and post-translational modification pathways. Our mESC RNA-interactome confirms RBPs recently found in HeLa and HEK293 studies and expands the atlas of RBPs with novel candidates, providing a valuable resource for the study of RNA-RBP networks in stem cells.

558-A Multifunctional interleukin-6 receptor aptamers

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An aptamer can form a defined tree dimensional structure which leads molecules to exhibit high affinities to inorganic or organic molecules. In our lab an RNA aptamer with high affinity (K_d = 20 nM) to the human interleukin 6 receptor (IL-6R) was selected. This aptamer triggers IL-6 receptor-mediated uptake without affecting IL-6R interactions with its natural ligands IL-6 and gp130. Furthermore, we were able to use the 19 nt long aptamer for cargo delivery into IL-6R presenting cells. The tree dimensional structure of the RNA aptamer was identified as an intrinsic all parallel quadruplex structure. The affinity of the aptamer to IL-6R was highly dependent on quadruplex structure formation and abolished by replacing one guanine being involved in quadruplex formation. Additionally, we could demonstrate that the IL-6R aptamer served as effective HIV *de novo* infection inhibitor by binding gp120 as primary target. Gp120 is presented on the surface of HIV particles and responsible for infection process. The presence of the aptamer during HIV *de novo* infection leads to prevention of infection due to blockade of gp120-CD4 complex formation. Structural similarities of IL-6R and gp120 could not be observed explaining the affinity of an aptamer to two different targets.

561-A Knock-out mice and HITS-CLIP reveal that the SGs assembly factor G3BP preferentially binds intron-retaining transcripts and controls their stability in the brain

Sophie Martin[1], Juan Gonzáles-Vallinas[2], Nicolas Bellora[2], Manuel Irimia[6], Latifa Zekri[3], Alexandra Metz[3], Karim Chebli[3], Michel Vignes[4], Eduardo Eyras[2], Javier F Caceres[5], Ben J Blencowe[6], Jamal Tazi[1]

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Stress granules (SGs) are non-membranous cytoplasmic foci formed as a cellular protective response to environmental stress, such as elevated temperature, oxidative stress, hypoxia, osmotic shock, UV irradiation, glucose deprivation or viral infection. SGs share common properties with other granules like processing bodies or neuronal transport granules. G3BP (RasGAP-SH3-domain Binding Protein) is a key factor involved in SGs assembly. To assess the physiological function of G3BP, we generated viable *G3bp1*-knockout (KO) mice, which demonstrated behavioral defects linked to the central nervous system (CNS) associated with ataxia phenotype. Immunohistochemistry pinpointed high expression of G3BP in the cytoplasm of hippocampal neurons and Purkinje cells of the cerebellum of wild-type (WT) mice. Also, electrophysiological measurements revealed that the absence of G3BP1 leads to an enhancement of short-term potentiation (STP) and long-term depression (LTD) in the CA1 area of *G3bp1* KO mice compared to WT mice. Consistently, G3BP1-deficiency in neurons leads to an increase in intracellular calcium and calcium release in response to (S)-3,5-Dihydroxyphenylglycine (DHPG), a selective agonist of group I metabotropic glutamate receptors. HITS-CLIP (High-Throughput Sequencing after Cross-Linking and ImmunoPrecipitation) experiments were carried out on WT and KO mouse brain to identify G3BP-associated RNAs. Surprisingly, many G3BP's targets turn out to be non-coding RNA sequences, essentially snoRNAs and intronic sequences. Interestingly, transcripts with retained introns appear to be enriched in the cerebellum compared to the rest of the brain. G3BP1 depletion leads to a decrease in the expression of these intronic sequences in the cerebellum. In particular, G3BP1 is essential to repress mature *Grm5* (metabotropic glutamate receptor 5) expression in the cerebellum by stabilization of the intron in its pre-mRNA. This study suggests a new mechanism of gene regulation, important in the cerebellum, based on stabilization of silenced premature RNA transcripts, which might be converted to mature transcripts and translated or targeted for degradation upon G3BP depletion.

564-A Sequence specific code in RRM–RNA interactions

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RNA–recognition motif (RRM) is a well known RNA interacting protein domain. RRMs are commonly present in all kingdoms of life and play a role at every stage of RNA life cycle: processing, splicing, editing, export, degradation and regulation of translation. Despite the large body of data available on sequence-specific RRM-RNA interactions, including numerous structures of RRM–RNA complexes, the "recognition code" is still not fully understood, and we cannot predict the RRM ligand specificity from its protein sequence alone. In this project we are using a multidisciplinary approach towards better understanding of RRM–RNA recognition. By combining bioinformatics (including sequence analyses, artificial intelligence methods, protein structure prediction, and protein–RNA docking) and biochemical and biophysical methods, we aim to provide the general understanding of the recognition code of RRMs in the evolutionary context. Thus far, comparative analyses of the RRMs and RRM–like sequences were used to build a database, where RRMs are classified into families based on sequence similarity. *In silico* analysis of RRM–RNA complexes, for which structure information is available, has been used to infer global trends in the formation of specific protein–RNA contacts. This is the starting point to assign substrate specificity and/or modulating affinity within the various RRM's families. Computational predictions of new RRM–RNA specificities are first tested *in vitro* and will be validated in human cell lines and submitted to structural studies. We have already started characterizing substrate specificity of novel RRMs with unknown mode of action using *in vitro* selection (SELEX). This approach revealed new interactions that are currently being further characterized. We aim to obtain information about specificity and build structural models of RRM-RNA structure for at least one member of each RRM family present in our database.

567-A Manipulating endogenous RNAs with synthetic RNA-binding proteins

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Post-transcriptional regulation of gene expression is ubiquitous and fundamental for the control of cell growth, differentiation and the complex developmental programs of multicellular eukaryotes. Because of their modular structure, repeat domain proteins are particularly well suited for these processes and have been widely adopted throughout evolution. We have expanded the RNA recognition code of Pumilio and FBF homology (PUF) proteins, enabling the design of RNA-binding proteins with programmable specificities. Furthermore, in recent work we have created synthetic proteins from another family of RNA-binding repeat domain proteins: the pentatricopeptide repeat (PPR) proteins. These artificial proteins have revealed the code for RNA binding by natural PPR domains and provide unique tools for manipulating endogenous RNAs. We show that designer RNA-binding proteins can be used to selectively manipulate the expression of endogenous mRNAs in human cells. The design of proteins that can bind any RNA sequence of interest and modulate its function will be important to elucidate the mechanisms by which genes are controlled at the RNA level and may provide potential therapeutics in the future.

References:

1. Filipovska A, Razif MF, NygÅrd KK, Rackham O. (2011) A universal code for RNA recognition by PUF proteins. *Nature Chemical Biology* 7(7): 425-427.

2. Filipovska A, Rackham O. (2012) Modular recognition of nucleic acids by PUF, TALE and PPR proteins. *Mol. Biosyst.* 8(3): 699-708.

3. Filipovska A, Rackham O. (2011) Designer RNA-binding proteins: new tools for manipulating the transcriptome. *RNA Biology* 8(6): 978-83.

570-A Architecture of catalytic complexes essential for synthesis and co-translational insertion of selenocysteine in humans

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Fundamental and highly conserved biological processes such as RNA transcription, posttranscriptional RNA modification, mRNA splicing, and protein synthesis are governed by complexes formed between proteins and nucleic acids. Upon formation of a particular binary, ternary or a higher order nucleoprotein complex, a protein or RNA catalyzes a reaction yielding a product critical for another biological process. Surprisingly, structural studies on these complexes revealed that the major principles of protein-nucleic acid recognition are often not universal and unified. Perhaps the least understood are the interactions stabilizing catalytic complexes required for synthesis, decoding and co-translational insertion of the 21st amino acid, selenocysteine. These processes, which are essential for the structure and function of selenoproteins and a number of vital cellular functions, can be divided into three general steps. In the first step, which is conserved in all organisms, seryl tRNA synthetase 'erroneously' attaches serine to selenocysteine tRNA (tRNA^{Sec}). Subsequently, a specific kinase (PSTK) and synthase (SepSecS) convert the seryl group into the selenocysteinyl moiety while being attached to tRNA^{Sec}. In the final step, a specialized elongation factor, EFsec, selects and delivers to the ribosome selenocysteinyl-tRNA^{Sec} where it facilitates decoding of the selenocysteine UGA codon. Although the process outline was drawn over a decade ago, it is still not understood how these enzymes recognize tRNA^{Sec} among other, more abundant elongator tRNAs. Herein, we present results from biophysical studies on various complexes from the human selenocysteine pathway. To determine the architecture of the individual complexes and to understand how tRNA^{Sec} is specifically recognized, X-ray crystallography, small angle X-ray scattering, multi angle light scattering, and analytical ultracentrifugation methods were employed. Our results provide the first comprehensive view onto the organization of the selenocysteine synthetic machinery in humans.

573-A HIV Nucleocapsid Protein Precursors are Effective Nucleic Acid Chaperone Proteins

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During HIV-1 maturation, Gag is processed by the viral protease at five cleavage sites. Three different forms of nucleocapsid protein, NCp15 (NCp9+p6), NCp9 and NCp7 appear successively in this process. Previous work has shown that the virus containing NCp15 shows greatly reduced infectivity, while the virus with NCp9 is still infectious.[1] These data suggest that NCp15 lacks one or more necessary functions during viral infection. Mature NCp7 is a nucleic acid (NA) binding and chaperone protein, involved in destabilizing and remodeling NAs during reverse transcription. We hypothesize that HIV-1 NCp15 may be a poor chaperone, which may explain why the virus containing NCp15 is not infectious. To test this idea, the chaperone activity of NCp7, NCp9 and NCp15 was compared. Gel-shift annealing assays show that NCp15 anneals tRNA to the primer-binding site at a similar rate as NCp7, whereas NCp9 is the most robust chaperone protein. Sedimentation assays to measure NA aggregation show a similar trend, i.e., NCp9>NCp15~NCp7. Mutating all 8 of the C-terminal acidic residues of NCp15 to Ala improves the annealing and aggregation activity of NCp15 to the level of NCp9. NMR chemical shift perturbation results suggest that in solution, the p6 domain of HIV-1 NCp15 folds back and interacts with the zinc fingers of NCp7 at residues F16, A25, K33 and K38. Our results suggest that this interaction slightly reduces chaperone function.

Taken together, our data show that HIV-1 NC precursors are effective chaperone proteins and differences in chaperone activity do not explain the requirement for NCp15 processing. We also determined that mutations in NCp15, designed to eliminate the interaction of the p6 domain with TSG101 or Vpr, failed to rescue viral infectivity. A recent study showed that HIV-1 virions containing NCp15 have irregular core structures,[2] which may affect reverse transcription and viral replication. It has also been reported that NCp15-NA complexes have altered morphology compared to NCp7-NA complexes,[3] which may explain the irregular cores and the requirement for NCp15 processing.

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1. Coren, L. *et al.* (2007) *J. Virol.* **81**, 10047-10054
2. De Marco, A. *et al.* (2012) *J. Virol.* **86**, 13708-13716
3. Mirambeau, G. *et al.* (2006) *J. Mol. Biol.* **364**, 496-511

576-A Crystal structure of the active conformation of the Shigella flexneri VapC toxin at 1.9 Å resolution

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Toxin-antitoxin (TA) loci have been found in all prokaryotic genomes that have been sequenced to date and typically encode two proteins, a toxin that inhibits cell growth and an antitoxin that upon binding its corresponding toxin, neutralizes its action [1]. The VapC toxins contain an N-terminal ribonuclease motif belonging to the PilT N-terminus (PIN) domain type, harbouring four highly conserved acidic residues known to be essential for metal ion coordination. The toxin VapC (MvpT) from the Gram-negative pathogen *Shigella flexneri* is capable of globally down-regulating translation by specifically cleaving initiator tRNAfMet in the anticodon region following release from a tight complex formed with the antitoxin, VapB [2]. We have previously determined the crystal structure of the VapC-VapB toxin-antitoxin complex from *Shigella flexneri* [3] and a structure of the isolated form of an archaeal VapC is also available [4].

Here, we present the first structure of the active form of VapC from a pathogenic bacterium. Recombinant VapC from *Shigella flexneri* harbouring an active site mutation was overexpressed in Escherichia coli, purified to homogeneity, crystallized and the structure was determined at 1.9 Å resolution by x-ray crystallography. Comparison with the antitoxin-inhibited structure from the VapC-VapB complex [3] reveals that VapC most likely is active as a dimer in which movements of two a-helices surrounding the active site create a more compact active site conformation upon release of the antitoxin.

1) Winther, K. S. & Gerdes, K. (2011). Proceedings of the National Academy of Sciences of the United States of America 108, 7403-7407.

2) Gerdes K, Wagner EG. RNA antitoxins. Curr Opin Microbiol. 2007;10:117–124.

3) Dienemann, C., Boggild, A., Winther, K. S., Gerdes, K. & Brodersen, D. E. (2011). Journal of molecular biology 414, 713-722

4) Bunker, R. D., McKenzie, J. L., Baker, E. N. & Arcus, V. L. (2008). Proteins 72, 510-518

579-A Responses of the mRNA interactome to genotoxic stress

Elisabeth Zielonka[1], Alfredo Castello[1], Benedikt Beckmann[1], Rastislav Horos[1], Anne-Marie Alleaume[1], Claudia Strein[1], Sophia Foehr[1], Bernd Fischer[1], Jeroen Krijgsveld[1], Matthias Hentze[1]

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RNA-binding proteins (RBP) play critical roles in stress responses like DNA damage through interactions with elements in functionally-related RNAs, called regulons. One of the most cytotoxic forms of DNA damage are DNA double strand breaks (DSB), which can cause mutagenic events or even cell death. This project focuses on the plasticity of the global RBP network (mRNA interactome) and RNA-regulons of mouse fibroblasts (NIH-3T3 cells) in response to genotoxic stress. Using interactome capture [1; 2] and high-resolution proteomics, we will define the RBP´s that respond during genotoxic stress. By further employing deep sequencing and crosslink-IP approaches, we will identify genotoxic stress RBP targets and assay their influence on cell survival. Taken together, this project aims to address key questions of the DNA damage response and the implication of RNA-regulons.

1) Castello A. et al., Cell 2012, 149 (6)

2) Castello A. et al., Nat Protoc. 2013, 8 (3)

RNP Structure, Function and Biosynthesis

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 582 A – 597 A
Location: Main Hallway & Sanada Foyer

582-A Dissecting RNA-binding protein complexes

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The cytoplasmic control of mRNAs is essentially facilitated via specific mRNPs expected to comprise transcript specific and dynamic subsets of RNA-binding proteins (RBPs). Some of these associate directly with the RNA via specific cis-determinants whereas others are expected to associate primarily via protein-association and form only transient and potentially unspecific contacts with targeted mRNAs.

The RNA-binding protein IGF2BP1 (IGF2 mRNA binding protein) was demonstrated to stabilize the c-myc (MYC) mRNA, presumably by associating with a cis-determinant in the coding region of the MYC transcript , the so called 'Coding Region instability Determinant (CRD)'. Moreover, the protein which is also termed ZBP1 (Zipcode Binding Protein 1) regulates the spatiotemporal control of b-actin mRNA (ACTB) translation and directs subcellular sorting of this transcript at least in primary cells. All these regulatory processes are presumed to be facilitated by a number of distinct mRNPs of unknown composition. To reveal how IGF2BP1-associated RBPs modulate the fate of ACTB and MYC mRNAs in tumor-derived cells we established an MS2-tethering approach using in vitro transcribed RNAs. This allows us to investigate which RBPs associate directly or indirectly with the analysed transcripts. If and how identified RBPs modulate the fate of MYC and/or ACTB transcripts is currently analysed by loss-of-function analyses (LOF) using shRNA-mediated knockdown.

585-A 5'-cytosine methylation of C2278 in 25S rRNA stabilizes 60S ribosomal subunit in yeast

Andriana Halacheva[1], *Martin Koš*[1]

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During ribosome biogenesis the eukaryotic ribosomal RNAs are extensively modified at more than 100 sites by methylation and pseudouridylation. The direct role of most of these modifications for either the biogenesis, stability or activity of the ribosome remains unclear. Among these modification is a highly conserved 5'-methylation of the cytosine 2278, located at the base of the helix 71 in the domain IV of the yeast 25S rRNA. The equivalent site is methylated in both eukaryotes and prokaryotes, however, its function is unknown. We have identified Rcm1p as the long elusive RNA methyltransferase of C2278 in yeast. Deletion of *RCM1* gene and/or snR75, snoRNA guiding 2'-O-ribose methylation of an adjacent G2288, leads to destabilization of 60S ribosomal subunit. SILAC analyses confirm partial loss of a group of ribosomal proteins located in the vicinity of the modified residues. SHAPE analyses confirm changes in the 25S rRNA conformation of the helix 71 and the surrounding. Our results show that the two studied modifications are important for stability of the rRNA structure and interaction with ribosomal proteins.

588-A Budding yeast telomerase RNA: Zooming in for more definition of a large RNA.

Nancy Laterreur[1], Isabelle Dionne[2], Jean-François Noël[2], Stephanie Larose[2], Raymund Wellinger[3]

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The RNA components of telomerases appear to be subject to extremely rapid evolution. For example, RNA transcription and maturation pathways vary widely and the RNA cannot easily be grouped with others. We showed that the budding yeast telomerase RNA, called Tlc1, can be classified as a snRNA. Not only is the 5'-TMG cap and a passage through the nucleolus fitting that model, we determined that the 3'-termination pathway is dictated by the classical non-coding RNA termination factors Nrd1 and Nab3 at sites near the mature polyA$^-$3'-end of the RNA. In order to understand its biology better, we also began investigating the transcriptional control of the Tlc1 RNA. Guided by phylogenetic comparisons, we discovered canonical SCB boxes in the promoter and a series of heterologous gene expression experiments demonstrate that these short sequence elements can be functional for directing cell cycle controlled transcription. Targeted mutagenesis of the SCB has functional consequences such as reduced levels of the RNA and short telomeres. Finally, using a tagged version of Tlc1 as target, RNA mediated ChIP experiments show a clear induction of transcription at the end of G1 of the cell cycle. We therefore hypothesize that the telomerase RNA, just as the RNA encoding Est1p, is made specifically at the entry of S-phase, together with a number of S-phase specific genes.

I will also present our investigations on a sub-structure in Tlc1 that has as of yet unknown functions. This approach, again based on phylogenetic comparisons, uncovered a new conserved substructure that we call CS2a on the stem-loop (SL) IVc. It is near an area shown to be involved in the binding of Est1p and therefore could be intimately associated with telomerase-telomere interactions. Indeed, targeting this new CS2a element by mutagenesis yielded evidence for loss of telomerase function *in vivo*. This effect can be explained by a loss of Est1-binding and therefore is Est1p-dependent. Surprisingly however, specific mutations that should only affect the predicted distal structure of SL IVc, but not Est1p-association, strongly affected catalytic telomerase activity. Using strains that carry a deletion of the *EST1* gene, we were able to conclude that this new function of the distal area in SL IVc we call TeSS, for Telomerase Stimulating Structure, indeed is Est1p-independent. I will present our description of the molecular functions of TeSS and hypothesize that this substructure may be a new commonality between all telomerase RNAs.

594-A Implication of the SMN complex in the biogenesis and steady state level of the Signal Recognition Particle

Nathalie Piazzon[1], Florence Schlotter[1], Suzie Lefebvre[4], Maxime Dodré[1], Agnès Mereau[2], Johann Soret[3], Aurore Besse[5], Martine Barkats[5], Rémy Bordonné[3], Séverine Massenet[1], Christiane Branlant[1]

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Spinal muscular atrophy is a severe motor neuron disease caused by reduced levels of the ubiquitous SMN protein. SMN is part of a complex that is essential for spliceosomal UsnRNP biogenesis. Signal Recognition Particle (SRP) is a ribonucleoprotein particle crucial for cotranslational targeting of secretory and membrane proteins to the endoplasmic reticulum. SRP biogenesis is a nucleo-cytoplasmic multistep process in which the protein components, except SRP54, assemble with 7S RNA in the nucleolus. Then, SRP54 is incorporated after export of the pre-particle into the cytoplasm. The assembly factors necessary for SRP biogenesis remain to be identified. Here, we show that 7S RNA binds to purified SMN complexes *in vitro* and that SMN complexes associate with SRP in cellular extracts, and we identified the RNA determinants required. Moreover, we report a specific reduction of 7S RNA levels in the spinal cord of SMN-deficient mice, and in a *S. pombe* strain carrying a temperature-degron allele of SMN. Additionally, micro-injected antibodies directed against SMN or Gemin2 interfere with the association of SRP54 with 7S RNA in X. *laevis* oocytes. Our data show that reduced levels of the SMN protein lead to defect in SRP steady-state level and describe the SMN complex as the first identified cellular factor required for SRP biogenesis.

597-A Dissecting the splicing-dependent mRNA binding of ASAP complexes

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The exon-junction complex (EJC) is a multi-protein complex, which is deposited on the mRNA by the spliceosome. It binds in a sequence-independent manner 20-24 nucleotides upstream of an exon-exon junction. The EJC core consists of four different proteins: eIF4A3, MAGOH, Y14 and Barentsz (Btz). This core complex serves as a binding platform for many associated proteins referred to as peripheral EJC components. Within the peripheral EJC, the proteins RNPS1, Acinus and SAP18 form a stable trimeric sub-complex, the ASAP complex. The recently solved crystal structure of the ASAP complex reveals how RNPS1, Acinus and SAP18 interact in vitro. However, it remains elusive how the ASAP complex assembles in living cells and which factors mediate its interaction with spliced mRNA and the EJC core.

We studied ASAP complex assembly in cell extracts with a combination of co-IP experiments and in vitro splicing assays. Using deletion mutants, we defined domains of RNPS1 and Acinus that are required for ASAP complex formation and mRNA interaction. Furthermore, we identified single point mutations of RNPS1, Acinus and SAP18 that partially or completely disrupt the ASAP complex and analyzed their capability to bind mRNA. Interestingly, formation of the trimeric complex is a prerequisite for the interaction with spliced mRNA and its bound protein complexes. The molecular basis of the interaction between ASAP complex and EJC is currently further characterized.

Taken together, our data suggest that interactions of EJCs and ASAP complexes contribute to the assembly of splicing-dependent mRNPs.

Riboregulation in Development

Date: Wednesday, June 12, 20:00 - 22:30
Abstract: 600 A
Location: Main Hallway & Sanada Foyer

600 A PX1 regulates protoxylem cell fate via RNA processing

600-A PX1 regulates protoxylem cell fate via RNA processing

Kamil Ruzicka[1], Ana Campilho[3], Sedeer El-Showk[3], Dominique Eeckhout[4], Geert De Jaeger[4], Jan Hejatko[2], Mikko Frilander[3], Yka Helariutta[3]

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Plant hormones cytokinin and auxin have been established as essential regulators of Arabidopsis vascular cell fate. Protein AHP6, a member of cytokinin transduction cascade, has been demonstrated to play a critical role during protoxylem development. In order to put AHP6 protein in a broader functional context, we made genetic screen, using *AHP6prom:GFP* as a marker of cytokinin activity in the protoxylem cells.

We isolated mutant *px1*, which shows a decreased *AHP6prom:GFP* activity, accompanied with aberrant protoxylem formation and altered hormonal responses. *PX1* codes for a weak allele of an embryonic lethal gene (EMB2016) which is, based on homology to known Drosophila proteins, involved in RNA processing, however with unclear function.

In order to elucidate the molecular role of EMB2016 complex, we made a tandem affinity purification (TAP) experiment. We identified homologs of EMB2016 interactors known from Drosophila, underlying the evolutionarily conserved character of the EMB2016 module, and mRNA adenine modifying enzyme, showing thus a novel functional link in this respect. An extensive phenotype analysis confirmed functional relevance of these interactions. In addition, transcriptional profiling revealed broad changes in auxin and cytokinin responsive genes expression in the *px1* genetic background.

In summary, we demonstrate the importance of adenine modifications in mRNA within the framework of hormonal regulations and developmental processes.

RNA Transport and Localization

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 603 A – 609 A
Location: Main Hallway & Sanada Foyer

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603 A **Assembly of TREX complex components on mRNAs**
606 A **Tetramolecular Fluorescence Complementation for Detection of Specific RNAs in Vitro**
609 A **mRNA transport and translation regulate neuritogenesis**

603-A Assembly of TREX complex components on mRNAs

Agnieszka M. Gromadzka[1], *Niels Gehring*[1]

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After their transcription in the nucleus, mRNAs are exported to the cytoplasm as ribonucleoprotein complexes (RNPs). Correctly processed mRNAs associate with a varying set of adaptor proteins that eventually direct the recruitment of the general export receptor NXF1. Although many studies have been carried out on export adaptors, the exact determinants for the interaction of the mammalian export machinery with export competent mRNAs remain unclear.

Here, we analyze the recruitment of the export factors UAP56, DDX39 and ALYREF using in vitro spliced mRNAs. Surprisingly, no preferential interaction of UAP56, DDX39 and ALYREF with spliced mRNAs was detected. While all three factors favor an association with the 5' end of spliced and non-spliced mRNAs, a contribution of exon junction complexes and 3' sequences to the RNA-binding of UAP56, DDX39 and particularly ALYREF could be detected. Hence, the cooperative interaction of export factors with exon junction complexes on mRNA with multiple introns may explain the preferential export of spliced mRNAs.

This study provides insights into the process of mammalian mRNA export and its dependence on splicing and the presence of exon junction complexes. Next, we aim to define the hierarchy of export adaptor recruitment leading to the efficient export of mammalian mRNAs.

606-A Tetramolecular Fluorescence Complementation for Detection of Specific RNAs in Vitro

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Asymmetric localization of mRNA is a mechanism to regulate gene expression spatially as well as temporally and contributes to many important developmental processes. To study localization mechanisms various probes for RNA imaging have been established. These probes can be nucleic acid- or protein-based and give a characteristic fluorescent signal upon binding to target RNAs. Still, nucleic acid-based probes–*e. g.* molecular beacons or forced intercalation probes–cannot be produced inside cells, complicating *in vivo* imaging.[1] Protein-based probes fused to split fluorescent reporters can be expressed by the cellular machinery. Sequence-specific binding of proteins to their target RNA and subsequent reconstitution of the fused fluorescent reporter allow RNA detection. However, the traditional fluorescent reporters based on split-GFP are large and self-assemble spontaneously, causing significant background.[2]

To circumvent these limitations we used a three-body split-GFP and developed a reporter system for detection of specific RNA *in vitro*.[3] This system consists of four components: two Pumilio variants each fused to just one ß-sheet of GFP, a GFP detector, and the target RNA, which triggers assembly of the whole complex (see figure 1 below). We used this system to differentiate between closely related RNAs after as little as 10 minutes with a background fluorescence of merely 1.4 %.[4] Since complex background of RNA and cell lysate did not prevent fluorescence complementation, detection of RNA with our system could become possible *in vivo*.

The ability to detect single stranded RNA sequence specifically might also be useful for detection of SNPs or discrimination of microRNAs. We currently extend our system to allow fast and easy detection of any given RNA of interest.

1 G. Bao, W. J. Rhee, A. Tsourkas, *Annu Rev Biomed Eng* **2009**, 11, 25-47.

2 Y. Kodama, C.-D. Hu, *BioTechniques* **2010**, 49, 793– 805.

3 G. S. Waldo, S. Cabantous (Los Alamos National Security, LLC), US 7,666,606 B2, **2010**.

4 S. J. Kellermann, A. K. Rath, A. Rentmeister, *Chembiochem* **2013**, 14, 200-204.

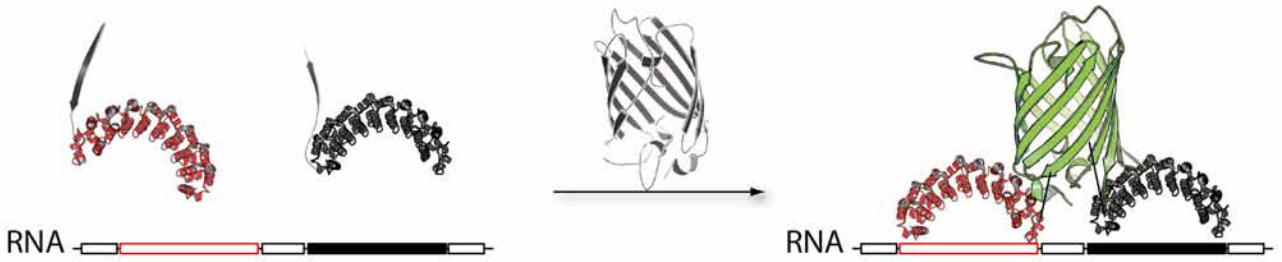

Figure 1: Tetramolecular Fluorescence Complementation (TetFC) for sequence-specific RNA detection. Binding of two Pumilio proteins to the target RNA at designated sites forms a complex that can recruit a GFP detector, thereby leading to fluorescence.

609-A mRNA transport and translation regulate neuritogenesis

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Post-transcriptional regulation of mRNA processing is involved in neural development and disease. Transport of mRNA and local translation in neurites are important mechanisms for neuron to control their development and function including neuritogenesis, synapse formation and synaptic plasticity. We have been focusing on two factors in neuronal RNA granules, the RNA-binding protein hnRNP Q1 and DEAD-box RNA helicase DDX3, to explore how they may regulate mRNA transport and translation during neuritogenesis. Using siRNA-mediated knockdown, we found that hnRNP Q1 and DDX3 could regulate neuritogenesis likely by regulating mRNA transport and translation, respectively, in primary cortical neurons and N2A cells. Knockdown of hnRNP Q1 increased neurite complexity in both primary cortical neurons and N2A cells. A search for mRNA targets of hnRNP Q1 identified functionally coherent sets of mRNAs involved in Cdc42-mediated cytoskeleton remodeling. Knockdown of hnRNP Q1 indeed reduced the level of several mRNAs encoding the components of the Cdc42/N-WASP/Arp2/3 pathway in neurites. This result suggests that hnRNP Q1 may participate in localization of mRNAs encoding Cdc42 signaling factors in neurites, and thereby regulate actin dynamics and control neuronal morphogenesis (Mol. Cell. Biol. 32, 2224, 2012). Knockdown of DDX3 inhibits neurite outgrowth in both cortical neurons and N2A cells. A screening of potential mRNA targets of DDX3-regulated translation identified functionally coherent genes involved in the Rac1-mediated signaling pathway. We demonstrated by using immunoblotting, immunostaining and quantitative RT-PCR that DDX3 knockdown reduced the level of Rac1 protein but not mRNA. Moreover, RNA pulldown and *in vitro* translation showed that DDX3 associated with the 5' UTR of Rac1 mRNA and regulated Rac1 translation in N2A cells. Furthermore, overexpression of constitutively active Rac1 could partially rescue neurite outgrowth defects in N2A cells. Therefore, our and others' studies indicate that functionally coherent mRNAs can be regulated by single proteins as mRNA regulons at the mRNA transport and translation levels in neurons. Cdc42 and Rac1 are critical molecules in regulating cytoskeleton remodeling and cell polarity. Identification of mRNA regulons encoding Cdc42 and Rac1 signaling factors may provide us insights into how they regulate neuritogenesis as well as build polarity in neurons.

RNAs in Diseases

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 612 A – 642 A
Location: Main Hallway & Sanada Foyer

612 A **Novel TAL1 targets beyond protein coding genes: identification of TAL1-regulated microRNA genes in T-cell acute lymphoblastic leukemia**

615 A **Ddx5/Ddx17 RNA helicases control multiple layers of gene expression during TGFß-induced Epithelial-to-Mesenchymal Transition (EMT)**

618 A **Rho Guanine Nucleotide Exchange Factor: A Novel RNA Binding Protein Involved in the Pathology of Amyotrophic Lateral Sclerosis**

621 A **MicroRNAs as lung cancer biomarkers**

624 A **Exploring the in vivo functions of the mammalian tRNA ligase**

627 A **Differential LMNA splicing leads to metabolic disorders**

630 A **Mutation of a Zinc Finger Polyadenosine RNA Binding Protein Causes Autosomal Recessive Intellectual Disability**

633 A **Hepatitis C virus induced up-regulation of miR-27 expression promotes hepatic triglyceride accumulation**

636 A **RNase MRP is involved in chondrogenic differentiation**

639 A **The RNA-binding protein Quaking critically regulates vascular smooth muscle cell phenotype**

642 A **FUS mutations strongly promote FUS-SMN and FUS-RNAP II interactions**

725-A **DNA-damage induced regulation of splicing of MDMX-mRNA in ovarian carcinomas**

612-A Novel TAL1 targets beyond protein coding genes: identification of TAL1-regulated microRNA genes in T-cell acute lymphoblastic leukemia

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The transcription factor TAL1 is downregulated early in T-cell development and frequently overexpressed in T-cell acute lymphoblastic leukemia (T-ALL). To identify a TAL1-dependent microRNA gene expression profile, we ectopically expressed TAL1 in the TAL1-negative T-ALL cell line P12 and performed low density array analysis. Initially, we identified eight microRNA genes whose expression changed significantly upon TAL1 overexpression. We then validated these results by quantitative PCR analysis after enforcing or silencing the expression of TAL1 in TAL1-negative and TAL1-positive T-ALL cell lines, respectively. This approach confirmed that miR-135a, miR-223 and miR-330-3p are upregulated by TAL1, whereas miR-146b-5p and miR-545 are downregulated. To assess the possibility that these microRNAs are direct targets of TAL1, we searched publicly available TAL1 ChIP-seq data (GSE29181) for the presence of TAL1 binding peaks up to 10kb upstream of the transcription start site (TSS) of each microRNA gene. We identified one peak in a putative promoter region for miR-146b, suggesting that this gene may be a transcriptional target of TAL1. Furthermore, two peaks can be observed upstream of miR-223 TSS. Because miR-223 has already been shown to play a pro-tumorigenic role in T-ALL, although not in the context of TAL1, we performed TAL1 ChIP-qPCR in JURKAT and CCRF-CEM cells using primers designed for the genomic areas covered by the two peaks in the miR-223 locus. We confirmed that upon TAL1 IP there is more than 2-fold enrichment in the amplified area within 3.5kbs upstream of the miR-223 TSS. These results indicate that miR-223 is a direct target of TAL1 in T-ALL. Interestingly, analysis of microRNA gene expression profiles in different T-ALL subsets revealed that TAL/LMO primary samples display higher levels of miR-223 ($p=0.03$) and tend to express lower levels of miR-146b-5p ($p=0.09$) than other T-ALL cases. Moreover, miR-223 and miR-135a appear to follow the same pattern of expression along normal human thymocyte development as TAL1, with high levels in CD34+ T-cell precursors and sharp downregulation in more differentiated subsets. In contrast, miR-146b-5p, which is negatively regulated by TAL1, is mostly expressed in mature, single-positive thymic subsets. Overall, our studies identify several TAL1 downstream micro RNA target genes, of which miR-146b and miR-223 may be directly regulated, and suggest that they may be components of TAL1 downstream regulatory networks involved in normal hematopoietic development and in T-ALL. Their actual participation in this malignancy and/or in TAL1-mediated physiological effects in hematopoiesis requires investigation. We are currently exploring the functional consequences of these microRNAs modulation in the development of the malignancy. We Expect to present these results in this congress.

615-A Ddx5/Ddx17 RNA helicases control multiple layers of gene expression during TGFß-induced Epithelial-to-Mesenchymal Transition (EMT)

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It is well known that Epithelial-to-Mesenchymal Transition (EMT) plays an important role in differentiation and is corrupted during tumorigenesis. TGFß is a potent inducer of EMT, through the activation of Smads, leading to transcription of EMT master genes (Snail, Zeb, Twist) that regulate epithelial and mesenchymal gene expression. Evidence shows that EMT result from regulation of gene expression programs at the post-transcriptional level (alternative splicing and miRNAs). Ddx5 has been reported to favor EMT and is known to be a transcriptional coactivator of Smads. Ddx5 and its paralog Ddx17 are also implicated in alternative splicing.

To characterize the role of Ddx5/17 during TGFß-induced EMT, we used the human mammary epithelial cell line MCF10A and showed that Ddx5/17 depletion by siRNAs inhibited the induction of EMT in response to TGFß. Ddx5 and Ddx17 interact with smad3 and act as smad3 co-activator to induced expression of EMT transcription factor snail1&2. Consequently under ddx5/17 depletion, TGFß no longer repressed the expression of E-cadherin and Ocludin, two proteins implicated in the cell-cell junction. Accordingly, cells are stilled attached and are not able to migrate. These results confirmed that Ddx5/17 are necessary for TGFß -induced EMT.

Paradoxically, we observed that Ddx5/17 endogenous expression decreased after 48 hours of TGFß treatment. We show that miR-181b is induced by TGFß in a Ddx5/17 dependant maner and target Ddx5/17 in a feedback loop control. Our hypothesis is that Ddx5/17 decreased expression contributes to the development of cellular programs during EMT. Indeed, Ddx5/17 depletion increased: 1- the expression of Zeb1, a EMT transcription factor and others mesenchymal markers in response to TGFß and 2- the formation of Actin stress fibers. Finally, using exon arrays, we show that Ddx5/17 participate to the regulation of alternative splicing events induced during EMT. Indeed Ddx5 and Ddx17 control alternative splicing of a large subset of genes including Enah, Stx16 and Fn1, genes relevant in the EMT process.

In conclusion, Ddx5 and ddx17 are necessary to initiate TGFß signaling, but their decreased expression in a second phase could contribute to the completion of the cascade of events leading to TGFß -induced EMT in particular at the splicing level. In this context Ddx5/17 control different steps of gene expression (transcription, miRNA and alternative splicing), to drive TGFß -induced EMT.

618-A Rho Guanine Nucleotide Exchange Factor: A Novel RNA Binding Protein Involved in the Pathology of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive disorder characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, protein aggregate formation in motor neurons, including neurofilamentous aggregates, is a neuropathological hallmark. Recent evidence supports the hypothesis that alterations in RNA metabolism in motor neurons can lead to the development of these aggregates. In mice, p190RhoGEF, a guanine nucleotide exchange factor, is involved in neurofilament protein aggregation in a RNA-triggered transgenic model of motor neuron disease. However, no information was known regarding the function of the human homologue of p190RhoGEF called Rho Guanine Nucleotide Exchange Factor (RGNEF) and its role in ALS. Here we studied the function of RGNEF and its involvement in the ALS pathology. We observed that RGNEF is an RNA binding protein that binds NFL mRNA. In addition, we demonstrated that RGNEF affects NFL mRNA stability via 3'UTR destabilization. The over-expression of RGNEF in a human cell line significantly decreased the level of endogenous NFL protein and its over-expression in yeast showed cytotoxicity comparable to TDP-43, another protein involved in ALS. When the pathology of RGNEF was analyzed we observed extensive cytoplasmic inclusions in ALS spinal motor neurons that co-localized with ubiquitin, p62/Sequestosome-1, TDP-43 and FUS/TLS (all of them known neurodegenerative markers). Finally, we investigated for genetic alterations performing sequencing of the gene that codifies for RGNEF (ARHGEF28). We found a new frameshift mutation and extensive regions of the gene with homozygosis in familial ALS cases. Our results provide further evidence that RNA metabolism pathways are integral to ALS pathology. This is also the first described link between ALS and a RNA binding protein with aggregate formation that is also a central cell signalling pathway molecule.

621-A MicroRNAs as lung cancer biomarkers

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Lung cancer is the leading cause of cancer-related death worldwide, with a 5- year survival rate of only ~15%[1]. The cause of this high mortality is due to the poor prognosis caused by a late disease presentation, tumour heterogeneities and limited understanding of tumour biology. Histologically, lung cancer is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is comprised of two most common subtypes, i.e., Squamous Cell Carcinoma (SQCC), Adenocarcinoma (ADC), and an additional type called Large Cell Carcinoma (LCC). With the emergence of targeted therapies directed against specific cellular alterations, an accurate classification became necessary[2]. In this context, the pattern of miRNAs expression could be useful in improving the classification of lung cancers and predicting their behavior. The relative quantification of miR-205 and miR-21 was reported by us and others to be a useful marker for differentiating subtypes of NSCLCs[3,4,5,6]. However, the majority of NSCLC patients have advanced disease stage at the time of diagnosis. It is therefore important to find diagnostic methods based on detection of early events. For this reason we compared the expression of miR-21 and miR-205 between cytological and histological samples to obtain a less invasive early method of diagnosis.

Besides ADC and SQCC, lung tumors include also a spectrum of neuroendocrine (NE) lesions with different histology, biology and dramatically different clinical behaviour, which include typical carcinoids (TC), atypical carcinoids (ATC), large cell neuroendocrine carcinomas (LCNEC) and small cell lung carcinomas (SCLC)[7]. In the present study we analyzed miR-375 and miR-192 expression to obtain a method to distinguish NE versus non-NE lung tumors and the different categories of NE lung tumors. By using a Support Vector Machine (SVM) algorithm we assessed whether, on the basis of miRNAs expression, it is possible to recognize different subtypes of lung cancers.

References:

[1] A. Jemal et al., *CA Cancer J Clin* **2009**, 59, p. 225-249.
[2] J.W. Neal, *Oncologist* **2010**, 15, p. 3-5.
[3] J.A. Bishop et al., *Clin Cancer Res* **2010**, 16, p. 610-619.
[4] D. Lebanony et al., *J Clin Oncol* **2009**, 27, p. 2030-2037.
[5] V. Del Vescovo et al., *Am J Surg Pathol* **2011**, 35, p. 268-7527.
[6] M. Barbareschi et al*., Am J Clin Pathol* **2011**, 136, p. 773-782.
[7] M. Barbareschi et al., *J Pathol* **1992**, 166, p. 343-350.

624-A Exploring the in vivo functions of the mammalian tRNA ligase

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Our laboratory has recently identified an elusive protein complex in human cells displaying tRNA ligase activity. The human tRNA ligase is a pentamer consisting of the catalytic subunit HSPC117, the DEAD box helicase Ddx1 and three proteins of unknown function - CGI-99, Fam98B and Ashwin. Only cells depleted of the essential subunit HSPC117 fail to complete tRNA splicing.

We envision additional roles for the mammalian tRNA ligase in RNA processing and RNA metabolic pathways, particularly given the more extensive functions of tRNA ligases in other organisms that include for example *hac1* mRNA splicing during the unfolded protein response as well as RNA repair. To elucidate the *in vivo* function of the mammalian tRNA ligase, we generated an HSPC117 knock-out mouse. Since the complete knock-out of HSPC117 leads to early embryonic lethality, we are currently focusing on the depletion of HSPC117 in the brain and cells of the immune system. Mammalian RNA ligation and RNA ligase enzymes are relatively unexplored. We hope that our mouse models will provide insights into the role of mammalian RNA ligation in diverse cellular functions, and potentially connect the failure of these functions to human disease.

627-A Differential LMNA splicing leads to metabolic disorders

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In humans, A-type lamins arise from *LMNA* gene by alternative RNA processing. The two main A-type lamins are lamin A and lamin C. Progerin is a truncated version of lamin A protein, involved in Hutchinson-Gilford progeria syndrome (HGPS), a premature aging disease. Most of the times, Progerin expression is due to a single splicing mutation in the exon 11 of *LMNA* gene (c.1824C>T; p.G608G).

Mutant mice carrying the equivalent HGPS splicing mutation in the *Lmna* gene (c.1827C>T; p.G609G) also accumulate progerin and phenocopy the main cellular alterations and clinical defects of HGPS patients. We demonstrate that changes in the splicing ratio between lamin A and progerin are key factors for lifespan since the $Lmna^{G609G/G609G}$ mice lived no longer than 5 months, whereas $Lmna^{G609G/+}$ mice lived up to one year. Strikingly, mice expressing only the lamin C isoform, due to targeted inactivation of lamin A (herein called $Lmna^{LCS/LCS}$ mice), lived longer than wild type mice. The initial characterization of $Lmna^{G609G/G609G}$ and $Lmna^{LCS/LCS}$ mice indicates that lamin isoforms expression influences adipose tissue homeostasis. We observed that progeria mice are lean compared to control mice whereas LCS mice become fat under chow diet. These modifications of body weight are correlated with an alteration of adipose tissue size without changes in food intake. Moreover oxymax analysis showed an increase in O2 consumption in progeria mice compared to the control mice, whereas LCS/LCS mice have a lower O2 consumption. Finally, microarray analysis of white adipose tissue revealed that a set of genes varies in opposite way between progeria and LCS mice. Interestingly, many of these genes are involved in energy metabolism. All these data showed that alterations in LMNA alternative splicing lead to a strong metabolic phenotype, characterized by a dysregulation of adipose tissue homeostasis.

630-A Mutation of a Zinc Finger Polyadenosine RNA Binding Protein Causes Autosomal Recessive Intellectual Disability

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Intellectual disability affects between 1-3% of people across the world. Patients with intellectual disability suffer from significantly subaverage intellectual function (IQ ≤70), which impinges on quality of life. In a collaborative effort, we have recently identified the first gene encoding a polyadenosine RNA binding protein, ZC3H14 (Zinc finger CysCysCysHis domain-containing protein 14), which is mutated in inherited nonsyndromic autosomal recessive intellectual disability. This finding uncovers the molecular basis for disease in these patients and provides strong evidence that ZC3H14 is essential for proper brain function. ZC3H14 is an evolutionarily conserved member of a novel class of tandem zinc finger (CCCH) polyadenosine RNA binding proteins. Studies of ZC3H14 orthologs in budding yeast and *Drosophila* provide insight into the role of this protein in post-transcriptional regulation of gene expression, specifically in the proper control of 3'-end polyadenylation of mRNA. Despite studies in yeast and *Drosophila*, functional characterization of ZC3H14 in vertebrates is crucial for understanding the role of this protein in brain function and the molecular mechanism underlying intellectual disability in these patients. Therefore, we have developed a conditional *ZC3H14* knockout mouse utilizing the Cre/loxP system to extend our studies to vertebrate ZC3H14 and address our hypothesis that ZC3H14 is required for proper expression of target mRNAs that are critical for neuronal function. We utilized the EIIa-Cre transgenic line, in which Cre-recombinase is expressed ubiquitously, to knockout ZC3H14 expression. EIIa-Cre +, ZC3H14 -/- mice are viable, suggesting that *ZC3H14* is not essential. These *ZC3H14* knockout mice provide us with an optimal model to study the requirement for ZC3H14 in higher order brain function. We are currently performing preliminary analyses to define molecular and functional effects of the loss of ZC3H14 by examining aspects of neuronal morphology, overall brain architecture, and cognitive behavior. Additionally, we will extend our observations from yeast and *Drosophila* and specifically investigate the role of ZC3H14 in 3'-end polyadenylation of mRNA for the first time in the mouse model organism. By understanding how ZC3H14 regulates target mRNAs—and thus their expression—in mice, we can begin to define the role of ZC3H14 in for normal brain function. Our long-term goal is to understand how dysregulation of post-transcriptional control of mRNA in neurons leads to neuronal dysfunction and consequently impaired brain function.

633-A Hepatitis C virus induced up-regulation of miR-27 expression promotes hepatic triglyceride accumulation

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MicroRNAs (miRNAs) are small RNAs that post-transcriptionally regulate gene expression. Their aberrant expression is commonly linked with diseased states, including hepatitis C virus (HCV) infection. To-date, there is a limited understanding of the biological relevance of HCV-induced changes in hepatic miRNA expression. Herein, we demonstrate that HCV replication induces the expression of miR-27 in cell culture and *in vivo* HCV infectious models. Furthermore, we establish that miR-27 overexpression in hepatocytes results in intracellular triglyceride accumulation and larger lipid droplets, as observed by triglyceride assays and coherent anti-Stokes Raman scattering (CARS) microscopy. This triglyceride accumulation coincides with miR-27 mediated down-regulation of host factors with known roles in triglyceride homeostasis, as measured by qRT-PCR. These repressed genes include PPAR-a, a transcription factor regulating the expression of genes associated with fatty acid catabolism, and ANGPTL3, an inhibitor of fatty acid uptake. We further demonstrate that treatment with a PPAR-a agonist, bezafibrate, is able to reverse the miR-27 induced lipid accumulation in Huh7 cells. This miR-27 mediated repression of PPAR-a signaling represents a novel potential mechanism of HCV-induced hepatic steatosis. This link was further demonstrated *in vivo* through the correlation between miR-27 expression levels and hepatic lipid accumulation in HCV infected SCID-beige/Alb-uPa mice. Lastly, overexpression of miR-27 was found to inhibit viral replication in hepatoma cells stably expressing full-length genomic HCV replicon. *Conclusion:* Collectively, our results highlight HCV's induction of miR-27 expression as a novel mechanism of steatosis, as well as the HCV therapeutic potential of miR-27.

636-A RNase MRP is involved in chondrogenic differentiation

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The ribonuclease mitochondrial RNA processing (RMRP) gene encodes the RNA component of a multi-protein-RNA complex called RNase MRP. This small nucleolar ribonucleoprotein particle is implicated in various cellular processes, including ribosomal biogenesis, mitochondrial RNA cleavage, cell cycle regulation and has been linked to telomerase. Mutations in the RMRP gene cause Cartilage Hair Hypoplasia (CHH). The phenotypic hallmark of CHH is dwarfism. We therefore hypothesized that RNase MRP is involved in chondrogenic differentiation of the growth plate during skeletal development.

To investigate the expression of RNase MRP during growth plate development RNase MRP protein subunits Rpp25, Rpp38 and Rpp40 were immunohistochemically detected in growth plates of 6 week old mice. Identical distribution patterns were observed: resting zone chondrocytes expressed RNase MRP proteins. Weak expression levels were observed in proliferative chondrocytes. Expression of RNase MRP proteins was clearly detectable in hypertrophic chondrocytes. This temporospatial expression was confirmed using *in vitro* culture models for chondrogenic differentiation. In chondrogenic ATDC5 and human bone marrow stem cells RMRP, Rpp25, Rpp30 and Rpp40 expression was upregulated from day 14 in differentiation onward, simultaneously with markers for chondrocyte hypertrophy. Chondrocyte proliferation and differentiation has shown to be tightly regulated by parathyroid hormone-related peptide (PTHrP), by preventing terminal differentiation of chondrocytes. We therefore investigated the effect of PTHrP on RMRP expression during chondrocyte differentiation in ATDC5 cells. PTHrP-mediated inhibition of chondrogenic differentiation displayed a dose dependent decrease in RMRP levels, indicating a relation between PTHrP, chondrogenic differentiation and RMRP expression. Interfering with RMRP expression using RMRP-specific siRNAs resulted in impaired chondrogenic marker expression. In addition, RMRP knockdown resulted in increased expression of early chondrogenic transcriptional regulator Bapx1/Nkx3.2, suggesting a regulatory role for RMRP in determining chondrocyte differentiation. Moreover, bone morphogenic protein-2, a known stimulator of hypertrophy, increases RMRP expression during chondrogenic differentiation of ATDC5 cells. Finally, transdifferentiation of CHH patient-derived dermal fibroblasts into chondrocyte-like cells indicated a more than 50% reduced Col10a1 expression in patient-derived fibroblasts as compared to control cultures.

In conclusion, our data indicate that RNase MRP is involved in chondrogenic development of the growth plate and appears to be predominantly associated with terminal hypertrophic differentiation. In addition, our data provide novel insight into the underlying molecular mechanism causing the CHH-associated skeletal phenotype.

639-A The RNA-binding protein Quaking critically regulates vascular smooth muscle cell phenotype

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Rationale: RNA-binding proteins are critical regulators of post-transcriptional RNAs, and can influence pre-mRNA splicing, mRNA localization, and stability. The RNA-binding protein Quaking (QKI) is essential for embryonic blood vessel development. However, the role of QKI in the adult vasculature, and in particular in vascular smooth muscle cells (VSMC), is currently unknown.

Objective: We sought to determine the role of the RNA-binding protein Quaking (QKI) in regulating adult VSMC function and plasticity.

Methods and Results: We identified that the RNA-binding protein Quaking (QKI) is highly expressed by neointimal VSMCs of human coronary restenotic lesions, but not in healthy vessels. In a mouse model of vascular injury, we observed reduced neointima hyperplasia in Qk^{v} mice, which have decreased QKI expression. Concordantly, abrogation of QKI attenuated fibroproliferative properties of VSMCs, while potently inducing contractile apparatus protein expression, rendering non-contractile VSMCs with the capacity to contract. We identified that QKI localizes to the spliceosome, where it interacts with the myocardin pre-mRNA and impacts the myocd_v3 / myocd_v1 balance. This shift in the transcriptional coactivation activity of Myocardin following arterial damage is tightly coupled with QKI expression levels.

Conclusions: We propose that QKI is a central regulator of VSMC phenotypic plasticity and that intervention in QKI activity can ameliorate pathogenic, fibroproliferative responses to vascular injury.

642-A FUS mutations strongly promote FUS-SMN and FUS-RNAP II interactions

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The motor neuron disease ALS has no known treatment and disease mechanisms are not understood. Previously, we characterized the interactome of the ALS-causative protein FUS and discovered that FUS associates abundantly with U1 snRNP and SMN, the protein deficient in the motor neuron disease spinal muscular atrophy (SMA). These and other data indicate that ALS and SMA share a molecular pathway (Yamazaki et al., Cell Reports 2012, 2:799). To gain insights into the function of FUS and its role in ALS, we have now carried out extensive analysis of the FUS interactome using FUS antibodies recognizing different epitopes. We found that FUS associates with RNAP II as well as several transcription-related proteins. To gain insights into the potential roles of these factors in ALS pathogenesis, we analyzed their interactions with FUS deletion mutants. As reported previously (Wang et al Nature 2008, 454:126), our data indicate that FUS forms an intramolecular interaction in which its N and C terminal regions bind to one another. We find a striking increase in the association of both SMN and RNAP II with FUS when it contains deletions that disrupt this intramolecular interaction. U1 snRNP binds with similar efficiency to full length FUS and the FUS mutants. Moreover, we find that the N-terminal prion-like domain and the C-terminal RGG-rich region show increased levels of binding to a number of proteins in nuclear extract. Thus, ALS-causing mutations in these regions of FUS could be pathogenic due to the increased interaction with SMN, RNAPII, and/or the proteins that we are in the process of identifying.

725-A DNA-damage induced regulation of splicing of MDMX-mRNA in ovarian carcinomas

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MDMX is an essential regulator of p53 activity during development and tumorigenesis. Among many different spliced transcripts known of MDMX, the variant MDMX-S has a prognostic relevance in different cancers. We opted to analyze the splicing mechanism underlying the generation of MDMX-S transcript. We also searched for a connection between the DNA-damage checkpoint kinases ATM and ATR with the alternative spliced MDMX-S transcript. The expression of MDMX splice variants, especially the ratio of FL-MDMX and MDMX-S was analyzed in 33 ovarian carcinoma samples. Furthermore, we investigated the role of ATM/ATR-pathway in the alternative splicing of exon 6 in an ovarian carcinoma cell line (OAW-42). RNA-Pull-Down was used to identify splice proteins which could be involved in the alternative splicing of exon 6. Finally, we knocked down a main NMD protein (Upf1) to see, whether MDMX-S is a target of the NMD.

The MDMX-S transcript was detected in 18 of 33 ovarian carcinoma samples. Increased level of MDMX-S compared with FL-MDMX associated with a decreased overall survival. We observed a significant increase of the MDMX-S transcript level upon treatment of OAW-42 cells with cisplatin. This could be prevented by inhibition of ATM/ATR and p53. Therefore, we conclude that DNA-damage pathways mediate the alternative splicing of the MDMX-mRNA. In addition, we identified two splicing proteins (PUF60 and hnRNP-C1/2) which seem to be involved in splicing of exon 6 of MDMX-mRNA. The inhibition of NMD by knock down of Upf1 resulted in the increase of the MDMX-S transcript level. This suggests that transcripts lacking exon 6 are subjected to degradation by NMD. In summary, according to the data presented here the MDMX-S transcript associates with an aggressive phenotype in ovarian carcinomas. However, since MDMX-S is subjected to degradation by NMD, the mechanism underlying this observed phenotype is still unknown.

*S.H. and A.W. contributed equally to the results of this study.

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Viral RNAs

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 645 A – 654 A
Location: Main Hallway & Sanada Foyer

645 A **Reduced HBsAg expression in occult HBV infection: alteration of a post-transcriptional regulatory mechanism?**
648 A **HMGA1 interaction with HIV-1 TAR modulates basal and Tat-dependent HIV transcription.**
651 A **Phosphorylation of hepatitis C virus RNA polymerase Ser29 and Ser42 by PRK2 regulates HCV replication**
654 A **DNA habitat and RNA inhabitants: Relevant questions of a qualitative RNA sociology**

645-A Reduced HBsAg expression in occult HBV infection: alteration of a post-transcriptional regulatory mechanism?

Daniel Candotti[1], *Jean-Pierre Allain*[2]

[1]National Health Service Blood & Transplant UK; [2]Department of Haematology, University of Cambridge, UK

Background and aims. Occult hepatitis B virus (HBV) infection (OBI) is defined as low plasma level of HBV DNA with undetectable HBV surface antigen outside the pre-seroconversion window period. Previous work suggested that some OBI might be associated with various mutations in genomic regulatory elements negatively affecting viral replication. Recent studies suggested that Pre-S2/S mRNA splicing might be essential for S protein expression. On the other hand, such splicing must be strongly controlled to prevent the accumulation of nonfunctional spliced S mRNA. The functional relationship between S mRNA splicing and S protein expression and the potential negative effect of specific mutations was investigated in OBI carriers.

Methods. The Pre-S2/S mRNA 5' splice donor site sequence of 176 OBI and 381 HBsAg+ control strains (genotype A-E) were analyzed. The influence of mutations on local RNA folding was evaluated using MFOLD program. The effect of selected mutations on splicing and S protein expression was tested by transient transfection experiments in Huh7 cells with cloned S gene with or without the distant HBV posttranscriptional regulatory element (PRE) of OBIs and controls, as well as mutated whole genome.

Results. In vitro S mRNA splicing was confirmed irrespective of HBV genotype and was effective whether an autologous or heterologous promoter controlled S gene transcription. Splicing positions seemed to be mainly conserved across strains but splicing variants were also found. Irrespective of HBV genotype, 51% of OBI sequences presented substitutions adjacent to the 5' splicing donor site compared to 31% of controls ($P<0.05$). These substitutions were predicted to disrupt a putative stem-loop structure in 54% of OBI variants. OBI strains appeared to be competent in S protein expression. However, the amount of HBsAg in culture supernatant was significantly lower in the majority of cells transfected with the S sequences from OBI strains when compared to HBsAg+ strains. Mutating the 5' donor site prevented splicing and resulted in significant reduction of HBsAg production. Similarly, the double mutation A453G/G463A observed in two OBI strains interfered with splicing and HBsAg production.

Conclusions. These preliminary data indicate that the sequence and context surrounding a 5' splicing site within the S gene is important for efficient HBV S mRNA expression. This region within the S gene appears to be a new post-transcriptional regulatory element in addition to PRE essential for the efficient expression of unspliced S mRNA among HBV strains.

648-A HMGA1 interaction with HIV-1 TAR modulates basal and Tat-dependent HIV transcription.

Sebastian Eilebrecht[1], Emmanuelle Wilhelm[2], Brendan Bell[2], Arndt Benecke[1]

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During transcription of the HIV genome the transactivating response element (TAR) located in the nascent viral transcript is playing a key role as a binding platform for host cellular co-factors as well as the viral transactivator of transcription (Tat). The Tat/TAR interaction is involved in the activation and recruitment of the host cellular positive transcription elongation factor b (P-TEFb), which subsequently releases the transcriptional block of RNA polymerase II in order to activate efficient viral transcription elongation.

We have recently identified the chromatin master regulator HMGA1 to interact with 7SK non-coding RNA in a regulatory fashion [1,2]. Here we show a highly specific interaction between HMGA1 and HIV-1 TAR, which leads to a reduction of HIV-1 promoter activity both in the absence and in the presence of Tat. HMGA1 competes with Tat for TAR binding and 7SK RNA competes with HIV-1 TAR for HMGA1 binding. These results support a model, in which HMGA1 blocks the binding of transcription activating factors --- such as Tat --- to TAR [3].

Thus, the interaction of HMGA1 with HIV-1 TAR might contribute to viral latency, which is the main reason for the inability of HAART to cure HIV infection.

References

1. Eilebrecht, S., Brysbaert, G., Wegert, T., Urlaub, H., Benecke, B.J. and Benecke, A. (2011) 7SK small nuclear RNA directly affects HMGA1 function in transcription regulation. *Nucleic Acids Res*, **39**, 2057-2072.

2. Eilebrecht, S., Becavin, C., Leger, H., Benecke, B.J. and Benecke, A. (2011) HMGA1-dependent and independent 7SK RNA gene regulatory activity. *RNA Biol*, **8**.

3. Eilebrecht S., Wilhelm E., Benecke B.-J., Bell B. and Benecke A. (2013) HMGA1 directly interacts with TAR to modulate basal and Tat-dependent HIV transcription. *RNA Biol*, **in press**.

651-A Phosphorylation of hepatitis C virus RNA polymerase Ser29 and Ser42 by PRK2 regulates HCV replication

Jae-Su Moon[1], Song-Hee Han[1], Jong-Won Oh[1]

[1]Yonsei Univeristy

Post-translational phosphorylation has important roles in regulating the structure and function of proteins and modulating protein-protein interactions for the rapid regulation of phosphosignaling pathways. In virus-infected cells, the function, stability, and subcellular localization of virus-encoded proteins can be altered by host kinase-mediated phosphorylation. Indeed, growing numbers of virus-encoded phosphoproteins have been reported recently and they are implicated in viral pathogenesis, virion assembly, and genome replication. For plus-strand RNA viruses, the viral RNA genome is replicated by virus-encoded RNA-dependent RNA polymerases (RdRps). RdRps of several plus-strand RNA viruses, including the hepatitis C virus (HCV), are phosphoproteins, and the phosphorylation of some viral RdRps has been shown to play an important role in viral replication.

The nonstructural protein (NS) 5B protein of HCV is the viral RdRp essential for viral RNA genome replication. We have previously shown that HCV viral genome replication is functionally linked to NS5B phosphorylation status regulated by the protein kinase C-related kinase 2 (PRK2). Here, we use a combination of biochemical studies and reverse genetics to show that the phosphorylation of HCV NS5B is required for viral RNA replication. Phosphoamino acid analysis of both *in vitro* and metabolically-labeled NS5B showed that PRK2 phosphorylates NS5B exclusively at the serine residues. By *in vitro* kinase assays using a series of deletions and site-specific mutants of NS5B and mass spectrometry analysis, we identified two PRK2 phosphorylation sites, Ser29 and Ser42, on the ?1 finger loop region of NS5B that extensively interacts with the thumb subdomain of NS5B. Colony-forming assays using drug-selectable HCV subgenomic RNA replicons revealed that the prevention of phosphorylation by Ala substitution at either Ser29 or Ser42 impairs HCV RNA replication. Furthermore, reverse genetics studies using HCV infectious clones with mutations at Ser29 or Ser42 showed that preventing phosphorylation resulted in the suppression of viral replication. Molecular modeling revealed that phosphorylation of NS5B stabilizes the interactions between the ?1 loop and the thumb subdomain, which are important for the formation of the closed conformation of NS5B known to be important for *de novo* RNA synthesis. Collectively, our results provide evidence that HCV NS5B phosphorylation has a regulatory role during the HCV RNA replication process.

654-A DNA habitat and RNA inhabitants: Relevant questions of a qualitative RNA sociology

Guenther Witzany[1]

[1]Telos - Philosophische Praxis

Background: Most molecular biological concepts result out of physical chemical assumptions on the genetic code that are basically more then 40 years old. Recent empirical data on genetic code compositions and rearrangements by mobile genetic elements and non-coding RNAs together with results of virus research and their role in evolution does not really fit into these concepts.

Results: If we look at the abundance of regulatory RNAs and persistent viruses in host genomes, the key players that edit genetic code of host genomes are consortia of interacting RNA agents and viruses that drive evolutionary novelty, integration into cellular DNA and regulation of cellular processes in all steps of development. There is increasing evidence that all cellular life is colonized by exogenous and/or endogenous viruses in a non-lytic but persistent lifestyle. They prefer cellular genomes as habitat and determine genetic host (group) identity.

Conclusions: If we take a step away from the mainstream molecular biology (including the physical chemical properties of quasispecies and mutant spectra) and systems biology (non-reductionistic physicalism) and move towards a more consortial thinking of cooperative ensembles of RNA stem loops, viruses, and subviral remnants of former infection events, we should move towards a kind of RNA sociology. Qualitative RNA-sociology emphasizes understanding of social interactions through analysis of active RNA sequences according contextual needs, i.e. the primacy of interactional motifs, that means functions prior to sequence syntax. In this respect the exaptations of former (infection derived) inventions to more appropriate cellular needs fit ideally into these investigations, that means the highly dynamic modular perspective on these interacting agents.

This talk will present some relevant questions of a qualitative RNA sociology that may complete molecular biological terms and methods.

Interconnections Between Gene Expression Processes

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 657 A – 669 A
Location: Main Hallway & Sanada Foyer

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657 A **Impact of specific RNA Pol II CTD phosphorylation patterns**
660 A **Paraspeckle formation during NEAT1 lncRNA biogenesis is integrated by the SWI/SNF chromatin remodeling complex**
663 A **Alternative splice variant of chromatin regulators drive specific transcription**
666 A **Double-stranded RNA-expressing plasmids selectively inhibit translation of exogenous mRNAs**
669 A **A NOVEL POLY(A) RNA-BINDING PROTEIN REGULATES A KEY SUBUNIT OF ATP SYNTHASE IN BREAST CANCER CELLS**

657-A Impact of specific RNA Pol II CTD phosphorylation patterns

Anne Helmrich[1], *Daniel Schümperli*[1]

University of Bern, Switzerland

The human RNA polymerase II (Pol II) largest subunit contains a C-terminal domain (CTD) with 52 Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (21 consensus and 31 non-consensus) repeats. Tyr1, Ser2, Thr4, Ser5 and Ser7 residues undergo dynamic phosphorylation and dephosphorylation throughout the transcription cycle in a gene specific manner. Whereas Serine modifications are known to orchestrate the binding of transcription and RNA processing factors to the transcription machinery, the impact of distinct phosphorylation combinations in guiding RNA-processing factors to specific genes remain to be investigated, as well as the role of consensus versus non-consensus heptapeptides.

To address these open questions, we perform in vitro phosphorylation studies and generate CTDs with defined modification patterns, either on a full-length 52 repeat or a shortened construct which lacks the non-consensus queue. Binding targets are identified in mass spectrometry approaches and localized by chromatin immunoprecipitation. Further in vivo studies will help understanding the distinct roles of specific CTD modifications.

660-A Paraspeckle formation during NEAT1 lncRNA biogenesis is integrated by the SWI/SNF chromatin remodeling complex

Tetsuya Kawaguchi[1], Akie Tanigawa[1], Takao Naganuma[1], Tetsuro Hirose[1]

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The paraspeckle is a unique subnuclear structure formed around the specific long noncoding RNA (lncRNA), NEAT1. NEAT1 lncRNA are comprised of two alternatively processed isoform transcripts, NEAT1_1 and NEAT1_2, in which the longer NEAT1_2 isoform is indispensible for paraspeckle formation (Sasaki et al., PNAS 2009). We recently identified 35 novel paraspeckle-localized proteins (PSPs) in which seven PSPs act as essential factors for paraspeckle formation through facilitating the steps required for the NEAT1 lncRNA biogenesis (Naganuma et al., EMBO J 2012). Here, we identified the SWI/SNF chromatin remodeling complexes as additional paraspeckle-localized factors that interacted with multiple essential PSPs. RNAi knockdown revealed that the intact SWI/SNF complex is required for the paraspeckle formation. This was supported by another observation that paraspeckle was undetectable in the SWI/SNF-deficient adrenal carcinoma cell line. What is the molecular mechanism underlying the function of the SWI/SNF complex in the parasepckle construction? Knockdown of the SWI/SNF components did not affect expression level of the essential NEAT1_2 isoform. Interestingly, the EU-pulse labeling experiment revealed that the transcript level of the newly synthesized nascent NEAT1_1 isoform was markedly increased in the SWI/SNF-depleted cells, although NEAT1_1 per se is dispensable for paraspeckle formation. We found that the association of the elongating form of RNA polymerase II (RNAPII) with phosphoserine 2 in the C-terminal domain was markedly increased in NEAT1_1 region and the most prominently increased at the NEAT1_1 terminator in the SWI/SNF-depleted cells. These data suggest that the SWI/SNF complex attenuates the elongation of RNA polymerase II (RNAPII) in the NEAT1_1 region. It raised an intriguing possibility that the optimized RNAPII elongation gives the proper environment for the assembly of various PSPs onto the nascent NEAT1 lncRNA which is required for the subsequent formation of the intact parasepeckle structure. We found that the RNA-protein and the protein-protein interactions between NEAT1 lncRNA and three essential PSPs were substantially weaken in the absence of SWI/SNF complex. We confirmed that the SWI/SNF complex interacted with RNAPII, the essential PSPs and NEAT1 lcnRNA. Taken together we argue that the SWI/SNF complex integrates the co-transcriptional ribonucleoprotein assembly onto the nascent NEAT1 lncRNA, which is required for the subsequent construction of the intact parasepckle, through controlling RNAPII elongation. Elucidation of the detail mechanism of the co-transcriptional assembly of ribonucleoprotein complexes onto NEAT1 lncRNA will tell us about the molecular basis underlying the fate determination of nuclear-retained lncRNAs distinct from that of mRNAs

663-A Alternative splice variant of chromatin regulators drive specific transcription

Oriane Mauger[1], *Eric Allemand*[1], *Christian Muchardt*[1], *Eric Batsché*[1]

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Both alternative splicing and epigenetic regulations are deregulated in pathogenesis. These two processes are connected and recent studies describe how chromatin-born information can participate in the regulation of the alternative splicing. However, the question of how the alternative splicing can influence the chromatin regulation is not well evaluated. Here, we present evidences describing the functions of splicing variants coding for well-characterized chromatin regulators: the histone methyl-transferases EHMT2 and Suv39H2, the Polycomb subunit EED, and the NURF subunit BPTF. We showed that, while these chromatin regulators are expressed in all the tissues we tested, the expression levels of the different isoforms is cell-type specific. In particular, the relative proportion of the different EHMT2 isoforms is specifically correlated with the epithelial-mesenchymal transition, suggesting that alternative splicing affects the activity of these proteins on cell differentiation.

In order to further investigate the function of the different isoforms generated by alternative splicing, we have followed their activity on the transcriptional regulation of known target genes. For this purpose, we have developed a new strategy of siRNAs design where the sequence of the siRNAs we use, base-pair with splice junctions and so, target only spliced process mRNA which is expected to reduce off-target effects. This allows us to deplete the cells from either all the species of mRNA or just designated splice variants. Inversely, we have also generated isoform-carried lentiviruses, allowing us to rescue the expression of some alternative spliced variants.

Our study reveals how alternative splicing regulation affects the function of chromatin regulator, which in turn, modulate the transcription of target genes.

666-A Double-stranded RNA-expressing plasmids selectively inhibit translation of exogenous mRNAs

Jana Nejepinska[1], *Radek Malik*[1], *Petr Svoboda*[1]

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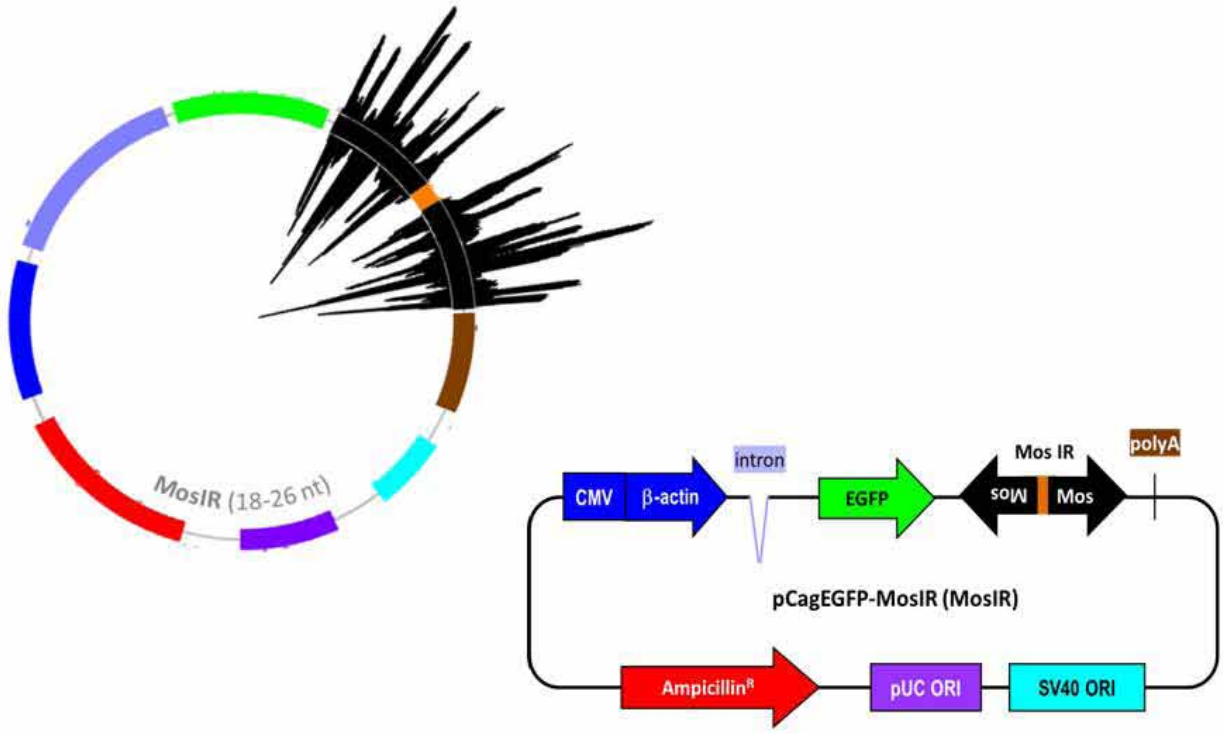
Double-stranded RNA (dsRNA) is linked to different effects in mammalian cells, including sequence-specific RNA interference, sequence-independent interferon response, and editing by adenosine deaminases. We have previously shown that long hairpin dsRNA expression in cultured cells does not necessarily activate the interferon response, it is poorly processed into siRNAs, and it is partially edited. Here, we demonstrate that dsRNA-expressing plasmids inhibit expression of transiently co-transfected reporter plasmids but they have a minimal impact on expression of endogenous genes or reporters stably integrated in the genome. The inhibition is concentration-dependent and independent of cell type, transfection method, or hairpin sequence. Deep sequencing data show low but detectable processing of hairpin RNA into small RNAs and preferential A-to-I editing of the dsRNA region (see figure 1 below). The inhibition likely occurs at the level of translation initiation and is mediated by the local and transient activation of protein kinase R. In conclusion, we demonstrate that expression from plasmids can be repressed if one of co-transfected plasmids generates dsRNA. We showed previously that the potential to generate dsRNA cannot be efficiently predicted and involves also commonly used plasmids. Moreover, our results indicate that cells can distinguish between endogenous and exogenous mRNAs and selectively inhibit the translation of foreign mRNAs in response to dsRNA.

Figure 1: Adenosine-to-inosine editing of small RNAs is specific to double-stranded RNA-forming region



669-A A NOVEL POLY(A) RNA-BINDING PROTEIN REGULATES A KEY SUBUNIT OF ATP SYNTHASE IN BREAST CANCER CELLS

Callie Wigington[1], Paula Vertino[2], Anita Corbett[1]

[1]Emory University, Department of Biochemistry; [2]Emory University, Winship Cancer Institute

Poly(A)-binding proteins, or Pabs, comprise one class of RNA-binding proteins that influence multiple steps in gene expression from polyadenylation within the nucleus to translation in the cytoplasm. All previously characterized Pabs interact with polyadenosine RNA via RNA Recognition motifs; however, a novel family of nuclear Pabs utilizes tandem CCCH zinc fingers for polyadenosine RNA recognition. The human member of this family of zinc finger Pabs is termed Zinc Finger CCCH-type containing #14 (ZC3H14) and has recently been linked to a form of non-syndromic intellectual disability as well as the estrogen receptor status of breast tumors. Although the function of ZC3H14 is unknown, the budding yeast counterpart, Nab2, ensures proper poly(A) tail length and mRNA export from the nucleus, suggesting a role for ZC3H14 in post-transcriptional regulation. In an effort to understand the post-transcriptional function of ZC3H14 as well as to gain insight into the spectrum of targets of nuclear Pabs, we employed a genome-wide microarray analysis of transcripts affected upon knockdown of the novel Pab, ZC3H14, or the well-characterized nuclear Pab, PABPN1. To focus on targets of relevance to breast cancer, experiments used MCF7 breast cancer cells. Knockdown of PABPN1 significantly affected ~17% of expressed transcripts as compared to knockdown of ZC3H14, which affected only ~1% of expressed transcripts. Results from this study show that PABPN1 affects the steady-state level of a large fraction of the transcriptome, which is consistent with the model that PABPN1 is a global polyadenylation factor. In contrast, knockdown of ZC3H14 affected a much smaller fraction of the transcripts, suggesting that ZC3H14 may have specific mRNA targets in these cells. One intriguing target that we selected for further analysis is the ATP Synthase F_o Subunit C, or ATP5G1. ATP Synthase is the central enzyme in oxidative phosphorylation and is responsible for the majority of ATP production in mammalian cells. The steady-state level of ATP5G1 is robustly decreased upon knockdown of ZC3H14, but not PABPN1. Interestingly, our data demonstrates that ZC3H14 specifically affects the steady-state mRNA level of ATP5G1 and not other ATP Synthase subunits, suggesting specificity. We are able to rescue the decrease in ATP5G1 by addition of a siRNA-resistant ZC3H14 plasmid. ATP5G1 is a rate-limiting component in F_o Subunit C assembly and ultimately ATP Synthase activity, and we observe that the effect of ZC3H14 on ATP5G1 results in decreased cellular ATP levels. Interestingly, upon RNA-IP of ZC3H14, we observe enrichment for ATP5G1 as well as other subunit C components, suggesting that ZC3H14 may partner with another factor to specifically regulate ATP5G1 levels. This data suggests that ZC3H14 may play a role in regulating cellular energy levels, which has broad implications for proper neuronal function as well as cancer.

RNA system biology

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 672 A – 678 A
Location: Main Hallway & Sanada Foyer

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672 A **The tissue specific and eco-responsive transcriptome of Drosophila**

675 A **Clindamycin ribosome interactions: a molecular dynamics study**

678 A **RNase disruption of transcriptional positive auto-regulation is essential for energy-efficient phenotypic switching**

672-A The tissue specific and eco-responsive transcriptome of Drosophila

James Brown[1], Nathan Boley[7], Robert Eisman[3], Michael Duff[6], Kenneth Wan[4], Ben Booth[4], Ann Hammonds[4], Carrie Davis[2], Lucy Cherbas[3], Piero Carninci[5], Thomas Gingeras[2], Peter Cherbas[3], Thomas Kaufman[3], Roger Hoskins[4], Brenton Graveley[8], Susan Celniker[4], Marcus Stoiber[1], Marlene Oeffinger[1], Peter Bickel[1]

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Integrative analysis of RNA sequencing data from *Drosophila melanogaster* reveals an animal transcriptome of unprecedented complexity, comprising discrete, tissue-specific transcripts. To decipher the information encoded in and probe the dynamics of the transcriptome, we generated and analyzed strand-specific poly(A)+ RNA-seq, CAGE, and cDNA sequences from 30 developmental time-course samples, 29 tissues, and 21 environmental perturbations in *D. melanogaster* which we call the Fly LifeMap. Although 55% of all genes and 66% of spliced genes may encode more than one protein isoform, a set of 47 largely neural-specific genes are the targets of RNA editing, extensive alternative mRNA splicing and have the potential to encode more than 1000 transcript isoforms each. The magnitudes of RNA splicing changes are much larger between tissues than throughout development or in response to environmental perturbations, and the majority of sex-specific splicing is gonad-specific. The gonads produce comparatively few protein isoforms per gene but express hundreds of previously unknown protein-coding and noncoding genes. The gonads also express antisense transcripts at key conserved developmental genes, including *eve*, *Dcr-2*, and *CTCF*. In neural tissue, antisense transcription is largely due to extension of 3'UTRs resulting in overlap with neighboring genes. In gonads, antisense transcription is due to independent transcription initiating at gonad-specific promoters. Most RNA-seq reads are accounted for within transcripts models, and we observe little evidence of pervasive transcription outside of primary transcripts. In summary, the *Drosophila* transcriptome is substantially more complex than previously recognized and arises from tissue-specific, combinatorial usage of well-defined promoter elements, splice sites, and polyadenylation sites.

675-A Clindamycin ribosome interactions: a molecular dynamics study

Katarzyna Kulczycka-Mierzejewska[1], *Joanna Trylska*[2], *Joanna Sadlej*[3]

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Antibiotics are drugs that treat the diseases of bacterial and fungal origin. Secondary uses include strengthening the immune system in cases of lowered immunity. Clindamycin is one of the antibiotics from the lincosamide class which are used to treat diseases caused mostly by Gram-positive bacteria and against some protozoal diseases. Lincosamidesinteract with the bacterial large (50S) ribosomal subunit and inhibit the process of protein synthesis leading to bacterial cell death. The increase of resistance of many bacterial strains against known antibiotics is caused by the expanded use of antibiotics in medical practice and veterinary. This is a very important reason for continuous work to find new, better and more effective antibacterial drugs.

Mutations of the antibiotic target are one of the common modifications that lead to bacterial resistance because such alterations typically prevent proper binding of the antibiotic in the targeted site. Currently, there are three structures of clindamycin in the complex with the 50S1,2,3 ribosomal subunit available in the Protein Data Bank coming from different organisms. Interestingly, two of the structures show significantly different conformations of the drug. The aim of this study was to compare the dynamic properties of the clindamycin binding site in the 50S subunit with and without the A2058G mutation to understand why this nucleotide substitution blocks the binding of lincosamides. To achieve this goal we applied full-atom molecular dynamics. Using the NAMD [4] package we performed four types of simulations: (1) the complex of clindamycin with the fragment of the 50S subunit of the ribosome as well as (2) the unbound ribosome fragment, (3) the complex of clindamycin with the mutated ribosome fragment and (4) the unbound mutated ribosome fragment.

To prepare the starting systems for the simulations we chose the 3OFZ 50S subunit structure from *Escherichia coli*, which consists of ribosomal RNA, ribosomal proteins, one clindamycin molecule, magnesium and zinc ions and crystal waters. For our simulations, we cut a sphere with the radius of about 20Å around clindamycin to account for the long-range interactions of the antibiotics in the 50S subunit. We added 228 K^+ ions to neutralize the charge and approximately 27000 TIP3P water molecules to solvate the system shaped in a truncated octahedron around the complex. The effect of the mutation on clindamycin positioning in the binding cleft resulting from these molecular dynamics simulations will be discussed.

678-A RNase disruption of transcriptional positive auto-regulation is essential for energy-efficient phenotypic switching

Elisabeth Wurtmann[1], Alexander Ratushny[2], Min Pan[1], Karlyn Beer[3], John Aitchison[2], Nitin Baliga[1]

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The distinct response kinetics of transcriptional and post-transcriptional regulation, and their non-uniform use across genes and environments, suggests that selective interplay of these mechanisms might be an evolved strategy to generate characteristic responses[1–4]. On the transcriptional level, the arrangement of transcription factors and their target genes into recurring network motifs generates response dynamics that are suited to particular information-processing functions[5]. Characteristic network topologies have also been observed at the post-transcriptional level with noncoding RNAs preferentially organized in feedforward loops[6]. Here, we have investigated whether the architecture of ribonuclease interactions in a gene regulatory network has any significant implication for environmental response strategies of an organism. We have discovered that RNases act in a characteristic regulatory motif that conserves energy by mediating efficient conditional phenotypic switching in both bacteria and archaea. Through analysis of the *E. coli* gene regulatory network, we made the intriguing observation that RNases preferentially target operons that are regulated by a transcriptional positive auto-regulation (PAR) loop, which is known to mediate bistable phenotypic switching[5]. A mathematical model for this regulatory motif predicted that relative to transcriptional repression, RNase-disruption of a PAR loop (RPAR) mediates significantly faster repression of target genes. Indeed, we find the RPAR motif to be essential for favorable bioenergetics of the salt-in strategy of salinity adaptation by extremely halophilic archaea. Specifically, the RPAR motif coordinates diverse metabolic functions to shift potassium uptake from a proton-gradient symport to a ATP-driven process in response to a wide array of environmental changes that alter membrane potential. Our results demonstrate that interplay of transcriptional and post-transcriptional regulation in a RPAR motif is a general principle by which prokaryotes regulate critical energy-consuming metabolic functions.

1. Shimoni, Y. *et al.* Regulation of gene expression by small non-coding RNAs: a quantitative view. *Mol Syst Biol* 3, 138 (2007).

2. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R.S. & Weissman, J.S. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. *Science* 324, 218–223 (2009).

3. Miller, C. *et al.* Dynamic transcriptome analysis measures rates of mRNA synthesis and decay in yeast. *Mol Syst Biol* 7, (2011).

4. Evguenieva-Hackenberg, E. & Klug, G. New aspects of RNA processing in prokaryotes. *Curr Opin Microbiol* 14, 587–592 (2011).

5. Alon, U. Network motifs: theory and experimental approaches. *Nat Rev Genet* 8, 450–461 (2007).

6. Beisel, C.L. & Storz, G. Base pairing small RNAs and their roles in global regulatory networks. *FEMS Microbiol Rev* 34, 866–882 (2010).

Bioinformatics

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 681 A – 696 A
Location: Main Hallway & Sanada Foyer

681 A **DNA methylation level is differentially correlated with the evolutionary features of coding exons in different genic positions**

684 A **EASANA: RNA-Seq and Affymetrix HTA2 data analysis, visualization and interpretation**

687 A **A new and systematic approach to analyse the population of sRNAs at the genome scale.**

690 A **DISCOVERING CONSERVED CIS-REGULATORY G-QUADRUPLEX MOTIFS IN THE TRANSCRIPTS OF HUMAN CHD8 GENE INVOLVED IN AUTISM**

693 A **cWords – systematic microRNA regulatory motif discovery from mRNA expression data**

696 A **PyCRAC CLIP data analyses predict a prominent role for Nrd1 and Nab3 in regulation of protein coding gene expression in yeast**

681-A DNA methylation level is differentially correlated with the evolutionary features of coding exons in different genic positions

Trees-Juen Chuang[1], Feng-Chi Chen[2], Yen-Zho Chen[1]

[1]Genomics Research Center, Academia Sinica; [2]Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes

DNA cytosine methylation is a central epigenetic marker. It is usually mutagenic, and may increase the level of sequence divergence. However, methylated genes have been reported to evolve more slowly than unmethylated genes. Hence, there is a controversy on whether DNA methylation is correlated with increased or decreased protein evolutionary rates. We hypothesize that this controversy has resulted from the differential correlations between DNA methylation and the evolutionary rates of coding exons in different genic positions. To test this hypothesis, we compare human-mouse and human-macaque exonic evolutionary rates against experimentally determined single-base-resolution DNA methylation data derived from multiple human cell types. We show that DNA methylation is significantly related to within-gene variations in evolutionary rates. First, DNA methylation level is more strongly correlated with C-to-T mutations at CpG dinucleotides in the first coding exons than in the internal and last exons, although it is positively correlated with the synonymous substitution rate in all exon positions. Second, for the first exons, DNA methylation level is negatively correlated with exonic expression level, but positively correlated with both nonsynonymous substitution rate and the sample specificity of DNA methylation level. For the internal and last exons, however, we observe the opposite correlations. Our results imply that DNA methylation level is differentially correlated with the biological (and evolutionary) features of coding exons in different genic positions. The first exons appear more prone to the mutagenic effects, whereas the other exons are more influenced by the regulatory effects of DNA methylation.

684-A EASANA: RNA-Seq and Affymetrix HTA2 data analysis, visualization and interpretation

Frédéric Lemoine[1], *Caroline Hégo*[1], *Olivier Ariste*[1], *Bertrand Coulom*[1], *Marc Rajaud*[1], *Pierre de la Grange*[1]

[1]**GenoSplice**

Recent advances in technologies allow to study the transcriptome as we never could before but also increase amount and complexity of data (up to several billion). Thus, analyzing such "big data" is challenging, time consuming, and needs the development of efficient and user-friendly tools. In this context, for public or private organization researchers, it is quite difficult to manage these new bioinformatics issues.

We developed a dedicated analysis and visualization tool for transcriptomics data named EASANA (publicly available at https://www.easana.com). This tool allows to display gene intensity taking into account the genomic context at the exon level. EASANA was first applied to Affymetrix Exon Array data (Jia et al., Cell 2012; Ameyar-Zazoua et al., Nat Struct Mol Biol 2012; Moreira et al., EMBO Mol Med 2013; Gandoura et al., J Hepatol 2013) and was recently improved to handle exon-exon junction intensity regulation information (Shen et al., Nucleic Acids Res. 2013). EASANA now enables the analysis and visualization of data from the last generation expression technologies. In particular, EASANA can now manage data from RNA-Seq experiments and from the last generation of splicing-sensitive microarray that include exon-exon junction probes (Xu et al., PNAS 2011): the Affymetrix HTA2 (Human Transcriptome Array 2.0).

EASANA handles all main types of RNA-Seq data (Illumina, SOLiD, 454…) and provide results at both gene and exon levels. For exon level analysis, EASANA analyzes regulation of alternative splicing as well as alternative first and terminal exons, known or not in publicly available gene annotations (i.e., new exon-exon junctions can be found). In addition, detection analysis of fusion transcripts and SNP/indel are also included.

A comparison between Illumina RNA-Seq and HTA2 using Human Muscle vs. Liver tissues will be presented: Detection sensibility, number of regulated genes and splicing pattern regulation will be detailed.

In March 2013, Affymetrix launched its new expression array named HTA2. As for RNA-Seq data, EASANA allows the analysis and visualization for data from this new generation array. Analyses at both gene and exon levels are provided and we currently develop algorithm to detect potential fusion transcripts from high-density expression microarray such as HTA2 (Affymetrix named GenoSplice as its preferred data analysis service provider for HTA2 data).

In addition to analyze genomics data, we provide help in the experimental design (e.g., number of replicates, sequencing depth), help in the biological interpretation of data (e.g., functional analysis of results), and follow-up until publication of results (scientific article and/or patent).

687-A A new and systematic approach to analyse the population of sRNAs at the genome scale.

Antonin Marchais[1], *Alexis Sarrazin*[1], *Arturo Marí Ordóñez*[1], *Olivier Voinnet*[1]

[1]ETHZ

The deep sequencing of small RNAs (sRNAs) provides a snapshot of RNA populations in the cells in specific conditions or tissues. In eukaryotes, these data usually reveal a complex mixture of miRNAs, siRNAs and degradation products, detected at different states of maturation or degradation, and mainly enriched for the sRNAs stabilised by their interaction with proteins or by their interaction with complementary RNAs. Despite the apparent noise created by the overlapping of these sub-populations of sRNAs, classical tools are able to characterize some of them using the rules of maturation described in the last decade. For instance, the sRNA sizes, the 2nt 3' overhangs and the 2D structures allow the detection of miRNAs, whereas the sizes, the 2nt 3' overhangs and their grouping along several hundred nucleotides defines siRNA clusters. These clusters occasional reveal phased sRNAs, illustrating DICER processivity.

In plants, the number of DICER and AGO proteins increases the complexity of this categorization and to bypass this problem we are forced to work with complex mutants. Moreover, the typical rules of sRNA maturation seem often insufficient to completely understand the observations.

By integrating tools classically used to discover correlations and periodicities in signal processing, our work focuses on the development of a new methodology to analyse in-depth the sRNA-seq data at the genome scale. The final goal is to achieve a more resolutive analysis with the current maturation rules and to discover new rules or signatures linked to the processivity of the DICERs and the known functional interactions between the proteins involved in silencing.

For instance in *A. thaliana*, RDR6 long double stranded RNA products are known to be mainly processed by DCL4 into 21nt siRNAs; from deep sequencing data analysis however, we also observed processing at a lower rate into 24nt siRNAs by DCL3. By the measure of cross-correlation of the genomic positions of these 21 and 24mers, our approach allowed us to propose a model for the ordered processing of these dsRNA substrates by both DICER proteins.

690-A DISCOVERING CONSERVED CIS-REGULATORY G-QUADRUPLEX MOTIFS IN THE TRANSCRIPTS OF HUMAN CHD8 GENE INVOLVED IN AUTISM

Emma Murray[1], Lawrence D'Antonio[2], Paramjeet Bagga[2]

[1]TAS-Bioinformatics, Ramapo College of New Jersey, Mahwah, New Jersey, USA; [2]Ramapo College of New Jersey

The human CHD8 (Chromodomain helicase DNA binding protein 8) is a chromatin remodeling agent. CHD8 represses transcription by enlisting the help of histone H1 at target genes where it remodels the chromatin. CHD8 binds to beta-catenin and suppresses the activity of p53/TP53-mediated apoptosis, thus negatively regulating the Wnt-signaling pathway. Mutations in the transcribed region of the human CHD8 gene have been linked to autism. Studying regulation of human CHD8 gene expression is expected to enhance our understanding of its function and role in human disease.

G-quadruplexes are highly stable three-dimensional structures formed in guanine rich DNA and RNA sequences. G-quadruplex structure consists of square coplanar arrays of G-tetrads. RNA G-quaduplexes have received significant attention because of their importance in biological processes such as regulation of protein synthesis and mRNA turnover.

The goal of this project has been to study the role of G-quadruplex forming motifsin regulating gene expression of human CHD8.

Using computational tools developed in our lab, we adopted a bioinformatics approach to map evolutionarily conserved G-quadruplexes in five orthologs of the humanCHD8 gene: chimpanzee, dog, bovine, mouse and rat. We discovered three highly conserved G-quadruplex motifs in the 3'-UTR of CHD8 mRNA. Two of these motifs were associated with microRNA target sites and one of them was found in close proximity to the polyadenylation signal. We found evolutionarily conserved G-quadruplex motifs in the 5 " flanking region of the alternatively transcribed human CHD8 isoform 1. Conserved G-quadruplexes were also found near the splice sites of alternative isoforms.

Our analysis suggests that conserved G-quadruplexes could potentially regulate translation efficiency, mRNA stability, and polyadenylation of the CHD8 mRNAs. Our results also suggest that G-quadruplexes could play a role in alternative transcription, as well as, alternative splicing.

693-A cWords – systematic microRNA regulatory motif discovery from mRNA expression data

Simon H. Rasmussen[1], *Anders Jacobsen*[2], *Anders Krogh*[1]

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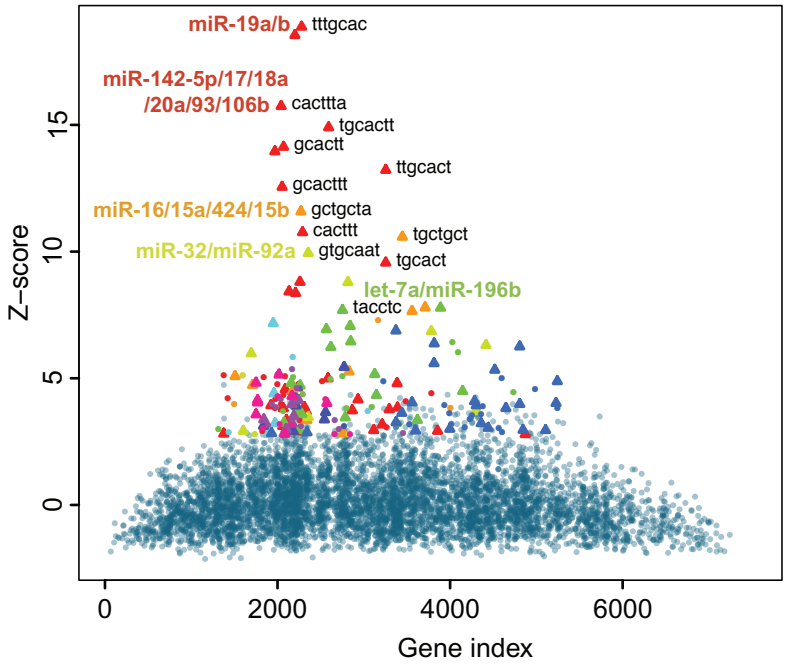
Background: Post-transcriptional regulation of gene expression by small RNAs and RNA binding proteins is of fundamental importance in development of complex organisms, and dysregulation of regulatory RNAs can influence onset, progression and potential treatment of many diseases. Post-transcriptional regulation by small RNAs is mediated through partial complementary binding to messenger RNAs leaving nucleotide signatures or motifs throughout the entire transcriptome. Computational methods for discovery and analysis of sequence motifs in high-throughput mRNA expression profiling experiments are becoming increasingly important tools for the identification of post-transcriptional regulatory motifs and the inference of the regulators and their targets.

Results: cWords is a method designed for regulatory motif discovery in differential case-control mRNA expression datasets. We have improved the algorithms and statistical methods of cWords resulting in at least a factor 100 speed gain over the previous implementation. On a benchmark dataset of 19 microRNA (miRNA) perturbation experiments cWords showed equal or better performance than two comparable methods, miREDUCE and Sylamer. We have developed rigorous motif clustering and visualization that accompany the cWords analysis for more intuitive and effective data interpretation. To demonstrate the versatility of cWords we show that it can also be used for identification of potential siRNA off-target binding. Moreover, cWords analysis of an experiment profiling mRNAs bound by Argonaute (AGO) ribonucleoprotein particles discovered endogenous miRNA binding motifs (Figure 1). The binding sites that occur in the mRNAs most bound by AGO as reported by cWords are shown in Figure 1A and these words correspond to the target sites of most expressed miRNAs in HEK293 cells, which are ranked in Figure 1B.

Conclusions: cWords is an unbiased, flexible and easy to use tool designed for regulatory motif discovery in differential case-control mRNA expression datasets. cWords is based on rigorous statistical methods that demonstrate comparable or better performance than other existing methods. Rich visualization of results promotes intuitive and efficient interpretation of data. cWords is available as a stand-alone Open Source program at Github https://github.com/simras/cWords and as a web-service at: http://servers.binf.ku.dk/cwords/.

A



B

miRNA	HEK293 expression	Seed site
miR-16	0.097	tgctgct
miR-196b	0.090	actacct
miR-15a	0.077	tgctgct
miR-142-5p	0.067	actttat
miR-19a	0.061	tttgcac
miR-18a	0.047	gcacctt
miR-17	0.042	gcacttt
miR-19b	0.041	tttgcac
miR-32	0.038	gtgcaat
miR-20a	0.033	gcacttt
miR-10a	0.031	acagggt
miR-424	0.026	tgctgct
miR-92a	0.023	gtgcaat
miR-15b	0.020	tgctgct
miR-93	0.018	gcacttt
let-7a	0.013	ctacctc
miR-30d	0.012	tgtttac
miR-106b	0.010	gcacttt
miR-140-5p	0.010	aaccact
miR-191	0.010	ttccgtt

Figure 1. **Word enrichment of AGO bound mRNAs.** (A) cWords word cluster plot showing 7mer 3'UTR words correlated with AGO binding in HEK293 cells. The top 10 words are annotated with IDs of the most abundant miRNAs in HEK293 cells. (B) Top 20 most abundant miRNAs in HEK293 cells, listing expression (relative clone frequency) and seed site (position 2-8) for each miRNA.

696-A PyCRAC CLIP data analyses predict a prominent role for Nrd1 and Nab3 in regulation of protein coding gene expression in yeast

Shaun Webb[1], Ralph D Hector[2], Grzegorz Kudla[4], Sander Granneman[3]

[1]Wellcome Trust Centre for Cell Biology, University of Edinburgh; [2]Institute for Structural and Molecular Biology, Centre for Synthetic and Systems Biology (SynthSys), University of Edinburgh; [3]Institute for Structural and Molecular Biology, Centre for Synthetic and Systems Biology (SynthSys), University of Edinburgh; [4]MRC Human Genetics Unit, University of Edinburgh

RNA-binding proteins play crucial roles in the synthesis, processing and degradation of RNA in a cell. To better understand the function of RNA-binding proteins, it is important to identify their RNA substrates and the sites of interaction. This would help to better predict their function and lead to the design of more focused functional analyses. The development of CLIP and related techniques has made it possible to identify direct protein-RNA interactions *in vivo* at nucleotide resolution.

The analysis of the high-throughput sequencing datasets, however, can be daunting, and demands more than a basic knowledge in bioinformatics and computer programming skills. The development of pyCRAC was driven by a requirement for a set of user-friendly, flexible and coherent set of tools tailored to handle CLIP and related RNA sequencing data. With a large number of valuable CLIP datasets now publically available, pyCRAC provides a comprehensive set of easy to use tools that allow the less experienced researcher to (fully) exploit these data.

To illustrate the functionality of pyCRAC, we have used the toolset to reanalyse recently published Nrd1 and Nab3 PAR-CLIP data, and we have also experimentally validated some of our findings. We found that Nrd1-Nab3 bound between 20 to 30% of protein-coding transcripts and we speculate that the proposed Nrd1-Nab3 "fail-safe" transcription termination mechanism is commonly used to prevent transcriptional read-through of mRNA coding genes. Nrd1 and Nab3 targets were also significantly enriched for enzymes and permeases involved in nucleotide/amino acid uptake and proteins involved in mitochondrial organization, indicating Nrd1-Nab3 function is tightly integrated with the nutrient response. Notably, transcripts encoding mRNA export and turnover factors were also frequently targeted. We conclude that Nrd1 and Nab3 play a prominent role in the regulation of protein coding gene expression, in particular genes involved in the nutrient response. Results from our *in silico* and experimental analyses will be presented.

Emerging & High-throughput Techniques for RNA

Date: Wednesday, June 12, 20:00 - 22:30
Abstracts: 699 A – 714 A
Location: Main Hallway & Sanada Foyer

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699 A **Comprehensive identification of RNA 5’ ends in E. coli**

702 A **Comparing the transcriptome of mouse and human using RNA sequencing**

705 A **Investigating ligation bias in small RNA library construction for high-throughput sequencing and the effect of different 3’ and 5’ adapters**

708 A **Dynamic responses of the hepatocytic mRNA interactome to metabolic reprogramming**

711 A **In vivo capture of RBPs bound to defined RNA species**

714 A **Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases**

699-A Comprehensive identification of RNA 5’ ends in E. coli

Ivana Bilusic Vilagos[1], *Bob Zimmermann*[1], *Meghan Lybecker*[1], *Nadia Tukhtubaeva*[1], *Renée Schroeder*[1]

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Recent prokaryotic transcriptome studies, led by advances in sequencing that have improved the technique's sensitivity, report antisense transcription to be more prevalent than previously expected. As many as 70% of annotated genes have been reported to have antisense counterparts that are regularly expressed at low levels. Although function of these widespread antisense transcripts is not known, they are often considered to be product of background or pervasive transcription.

We adapted and modified the standard RNA-seq library preparation method in order to discriminate between pervasive transcription products and stable, likely regulated, transcripts with a defined 5' end. The modification of the library preparation allowed us to specifically enrich for 5' ends resulting in most comprehensive overview of *E. coli* transcripts featuring stable 5' ends. Moreover, we were able to distinguish between primary transcriptional start sites (TSSs) with 5' triphosphate and stable 5' ends resulting from ribonuclease processing, bearing a monophosphate. We identified alternative TSSs of known genes as well as novel TSSs giving rise to unexpected short, mostly antisense RNAs. In addition, we monitored the influence of well-known RNA chaperone Hfq on TSSs profile. Expression of novel short RNAs, as well as their dependence on Hfq were confirmed by Northern blot analysis, further supporting the sequencing data. Overall, our data suggests strands opposite to annotated genes have potential to code for regulated RNAs with discrete 5' ends.

702-A Comparing the transcriptome of mouse and human using RNA sequencing

Allissa Dillman[1], Melissa McCoy[1], Marcel van der Brug[2], Mark Cookson[1]

[1]National Institutes of Health; [2]Diagnostics Discovery Department, Genentech, Inc.

Mice are often used as model organisms for human disease. There are many cases, however, in which mouse models do not recapitulate all of the symptoms present in the relevant human disease. This is especially true in models of neurological disease. The brain is an incredibly complex organ and understanding what makes the human brain unique and what it shares with its rodent models may help us to understand why some models are have better face validity than others. RNA sequencing (RNA-Seq) is uniquely applicable for direct comparison of gene expression between species as it does not require the use of sequence-specific probes, as is the case with microarray. We have used RNA-Seq to create a high-resolution transcriptome data set of mouse cerebral cortex and human frontal cortex. We found a large number of genes more highly expressed in one species as compared to the other. We validated a number of these findings using independent techniques. Taking advantage of the sequencing information found with RNA-seq we also noted that conserved A-to-I RNA editing sites were nonsynonymous coding changes in all but one case. We propose this dataset as a helpful resource for interrogating gene expression differences between humans and mice.

705-A Investigating ligation bias in small RNA library construction for high-throughput sequencing and the effect of different 3’ and 5’ adapters

Ryan Fuchs[1], *Zhiyi Sun*[1], *Fanglei Zhuang*[1], *G. Brett Robb*[1]

[1]New England Biolabs

High-throughput sequencing (HTS) has become a powerful tool for the detection of and sequence characterization of microRNAs (miRNA) and other small RNAs (sRNA). Unfortunately, the use of HTS data to determine the relative quantity of different miRNAs in a sample has been shown to be inconsistent with quantitative PCR and Northern Blot results. Several recent studies have concluded that the major contributor to this inconsistency is bias introduced during the construction of sRNA libraries for HTS and that the bias is primarily derived from the adapter ligation steps; specifically when single stranded adapters are sequentially ligated to the 3' and 5'-end of sRNAs using T4 RNA ligases. In our current study we investigate the effects of ligation bias by using a defined mixture of 962 miRNA sequences and several combinations of adapters in HTS library construction. Our results provide insight about the nature of ligation bias and allowed us to design adapters which reduce ligation bias and produce HTS results that more accurately reflect the actual concentrations of miRNAs in the defined starting material.

708-A Dynamic responses of the hepatocytic mRNA interactome to metabolic reprogramming

Rastislav Horos[1], Alfredo Castello[1], Bernd Fischer[1], Katrin Eichelbaum[1], Sophia Foehr[1], Anne-Marie Alleaume[1], Claudia Strein[1], Benedikt Beckmann[1], Jeroen Krijgsveld[1], Matthias Hentze[1]

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mRNAs are regulated at multiple steps throughout their lifetime by RNA-binding proteins (RBPs) which collectively represent the "mRNA interactome" of a cell. Interestingly, many unexpected proteins such as metabolic enzymes were found to bind mRNAs in vivo [1, 2], potentially playing important roles interconnecting posttranscriptional regulation with cellular metabolism [3]. IRP1/aconitase represents a well studied example, which, depending on intracellular iron levels, switches between functions as an enzyme or an RBP controlling mRNAs involved in iron metabolism. As liver cells represent a metabolically critical cell type, we determined the mRNA interactome of hepatocytic HuH-7 cells by in vivo RNA-protein crosslinking, isolation of mRNP complexes by oligo-d(T) chromatography and analysis by quantitative mass spectrometry [4]. We identified 726 proteins, including known RBPs, expected RBPs that are expressed in the hepatic cells, and many previously unknown RBPs. Interestingly, especially enzymes of central energy metabolism pathways display mRNA binding, notably of the glycolytic pathway and the TCA cycle. Next, cells were treated with the glycolytic inhibitor 2-deoxyglucose or left untreated prior to interactome capture and comparative quantitative proteomic analysis. We will describe the responses of the HuH-7 cell mRNA interactome to altered glycolysis, and report the development of comparative, quantitative interactome capture as an informative experimental approach to characterize the states and responses of cellular systems.

1. Castello, A., et al., Insights into RNA Biology from an Atlas of Mammalian mRNA-Binding Proteins. Cell, 2012. 149(6): p. 1393-406.

2. Baltz, A.G., et al., The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol Cell, 2012. 46(5): p. 674-90.

3. Hentze, M.W. and T. Preiss, The REM phase of gene regulation. Trends Biochem Sci, 2010. 35(8): p. 423-6.

4. Castello, A., et al., System-wide identification of RNA-binding proteins by interactome capture. Nat Protoc, 2013. 8(3): p. 491-500.

711-A In vivo capture of RBPs bound to defined RNA species

Birgit Schuster[1], *Alfredo Castello*[1], *Benedikt Beckmann*[1], *Bruno Galy*[2], *Matthias Hentze*[1]

[1]European Molecular Biology Laboratory (EMBL), Germany; [2]Molecular Biology Laboratory (EMBL), Germany

Recently, a comprehensive and unbiased method, called mRNA interactome capture, was developed for global identification of RBPs in living cells [1; 2; 3]. The design of this method focuses on determination of complete mRNA interactomes, but cannot be applied to capture RBPs assembled on defined RNA species. Methods to identify RNAs bound by single RBPs were successfully setup before (CLIP, PAR-CLIP, HITS-ClIP [reviewed in 4]). However, identifying RBPs that bind a particular RNA has been challenging so far. Here we present an experimental approach to determine all RBPs bound to a defined RNA species *in vivo,* which should enable studying the plasticity of particular Ribonucleoproteins (RNPs) throughout the different steps of RNA life, under changing cellular conditions (e.g. stress and disease) or different cellular stages. Using the global mRNA interactome capture approach including *in vivo* UV crosslinking as a starting point, we tailor the pull-down protocol by making use of DNA/LNA mixmer oligonucleotides as specific bait. Our proof of principle model is based on luciferase reporter constructs that allow introducing known RNA motifs upstream or downstream of the targeted open reading frame. We will discuss performance parameters of our approach to meet an urgent need in RNA research.

[1] Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, Strein C, Davey NE, Humphreys DT, Preiss T, Steinmetz LM, Krijgsveld J, Hentze MW. Cell. 2012; 149 (6)

[2] Baltz AG, Munschauer M, Schwanhäusser B, Vasile A, Murakawa Y, Schueler M, Youngs N, Penfold-Brown D, Drew K, Milek M, Wyler E, Bonneau R. *et al.* Mol. Cell 2012 46 (5)

[3] Castello A, Horos R, Strein C, Fischer B, Eichelbaum K, Steinmetz LM, Krijgsveld J, Hentze MW. Nature Protoc. 2013; 8 (3)

[4] Milek M, Wyler E, Landthaler M. Semin. Cell Dev.Biol. (2012) 23 (2)

714-A Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases

Migle Tomkuviene[1], *Beatrice Clouet-d'Orval*[3], *Elmar Weinhold*[2], *Saulius Klimasauskas*[4]

[1]Vilnius University Institute of Biotechnology, Vilnius, Lithuania; [2]Institute of Organic Chemistry, RWTH Aachen University, Aachen, Germany; [3]Laboratory of Microbiology and Molecular Genetics, CNRS-UMR 5100, Toulouse, France; [4]Vilnius University, Institute of Biotechnology, Department of Biological DNA modification

Biophysical and mechanistic investigation of RNA function requires site-specific incorporation of spectroscopic and chemical probes. In nature, a particular class of RNA methyltransferases, box C/D ribonucleoprotein complexes (C/D RNPs), direct AdoMet-dependent site-specific 2'-O-methylation to numerous biological sites [1]. Precise base pairing of a guide RNA and the substrate selects the target nucleotide (Fig. 1A). We have combined the specificity of C/D RNP machinery with synthetic AdoMet analogs to incorporate a reactive group which could be further appended with a desired label (Fig. 1B and 1C). Therefore we have *in vitro* reconstituted a C/D RNP from the thermophilic archaeon *Pyrococcus abyssi* and demonstrated its ability to transfer a prop-2-ynyl group from a synthetic cofactor analog [2] to both the wild-type and newly programmed target sites in model tRNA and pre-mRNA molecules. Target selection of the RNP was programmed by changing a 12 nt guide sequence in a 64-nt C/D guide RNA leading to efficient derivatization of three out of four new targets in each RNA substrate. We also show that the transferred terminal alkyne can be further appended with a fluorophore using a bioorthogonal azide-alkyne 1,3-cycloaddition (click) reaction (Fig. 2). The described approach [3] for the first time permits synthetically tunable sequence-specific labeling of RNA with single-nucleotide precision.

References:

[1] Reichow, S.L. et al. (2007) The structure and function of small nucleolar ribonucleoproteins. Nucleic Acids Res., 35(5), 1452–1464.

[2] Willnow, S. et al. (2012) A selenium-based click AdoMet analog for versatile substrate labeling with wild-type protein methyltransferases. ChemBioChem, 13:8, 1167–1173.

[3] Tomkuviene, M. et al. (2012) Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases. Nucleic Acids Res., 40(14), 6765-73.

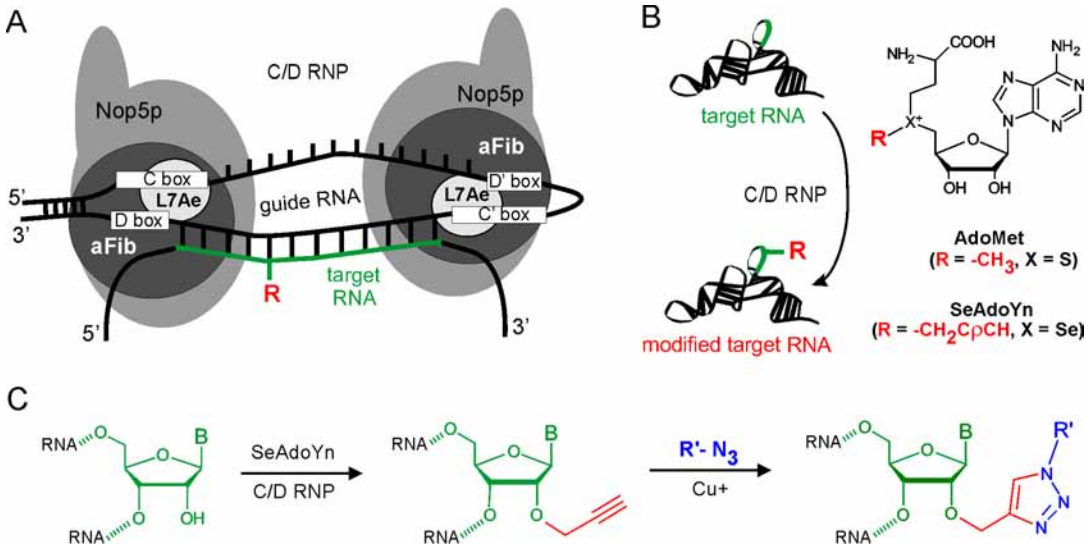
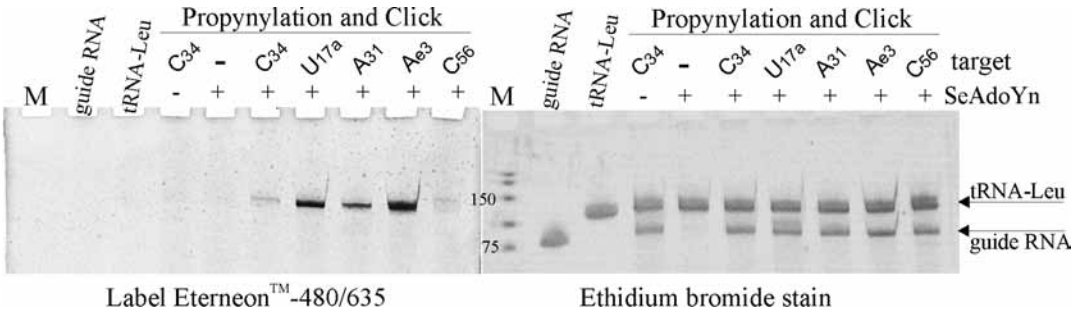


Figure 1. Archaeal C/D RNP-directed sequence-specific modification and labeling of target RNA. (**A**) Schematic structure of a C/D RNP complex with substrate RNA. Core proteins L7Ae, Nop5p and aFib are bound at the C/D and C'/D' sites of a guide RNA. One of the variable guide sequences is shown base-paired to a target sequence (green) of a substrate RNA. Modification occurs at a nucleotide complementary to the 5th position upstream from the D box; (**B**) C/D RNP-directed transfer of an activated side chain (red) from a cofactor S-adenosyl-L-methionine (AdoMet, X=S and R=methyl) or its analog SeAdoYn (X=Se and R=prop-2-ynyl) onto an RNA substrate; (**C**) Two-step labeling of target RNA via a C/D RNP-directed alkynylation, followed by Cu(I)-assisted click-coupling of a fluorophore azide (blue).

Figure 2. C/D RNP-dependent fluorescent labeling of predetermined sites in a model tRNA substrate via guide RNA-directed enzymatic



propynylation and click-coupling of an Eterneon(480/635) azide.