



SWISS RNA WORKSHOP

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9:15-18:20h

Universität Bern, UniS, Schanzeneckstr. 1, Hörsaal A003

Organizers: Angela Krämer, Oliver Mühlemann
and Daniel Schümperli

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Opening remarks (9:15)

Angela Krämer, Department of Cell Biology, University of Geneva
Daniel Schümperli, Institute of Cell Biology, University of Bern

Splicing and disease (9:25-10:40)

Analysis of pre-mRNA splicing in cell lines derived from patients with retinitis pigmentosa and dominant mutations in pre-mRNA splicing factor genes *PRPF31*, *PRPF8* and *PRPF3*

Goranka Tanackovic (C. Rivolta)
Department of Medical Genetics, University of Lausanne, Switzerland

SR, hnRNP, PKC and EJC: Converging complexity to control the splicing of the apoptotic regulator Bcl-x

Benoit Chabot
Département de microbiologie et d'infectiologie, Université de Sherbrooke, Québec, Canada

In vivo splicing modulation rescues a severe mouse model for Spinal Muscular Atrophy

Kathrin Meyer (D. Schümperli)
Institute of Cell Biology, University of Bern

Coffee break and setting up of posters (10:40-11:20)

Structures of RNPs (11:20-12:00)

NMR structure of the tra2 β 1 splicing factor in complex with RNA revealed an unexpected extensive interaction with the C-terminal extremity of the RRM

Antoine Cléry (F. Allain)
Institute for Molecular Biology and Biophysics, ETH Zürich

An assembly chaperone collaborates with the SMN-complex to generate spliceosomal snRNPs

Ashwin Chari (U. Fischer)
Department of Biochemistry, University of Würzburg, Germany

Nuclear regulation (12:00-12:40)

Trans-acting antisense RNAs mediate transcriptional gene cosuppression in *S. cerevisiae*

Nissrine Beyrouthy (F. Stutz)
Department of Cell Biology, University of Geneva

Yeast telomere repeat containing RNA (TERRA) and telomere length regulation

Brian Luke (J. Lingner)
ISREC, Epalinges

Lunch and poster session (12:40-14:10)

MicroRNAs and RNA binding proteins - control of translation and mRNA stability (14:10-16:05)

Stress-dependent coordination of transcriptome and translome in yeast

André P. Gerber
Institute of Pharmaceutical Sciences, ETH Zürich

Translational control by miRNAs and RNA-binding proteins

Matthias W. Hentze
European Molecular Biology Laboratory, Heidelberg, Germany

Function and regulation of the *let-7* microRNA

Helge Grosshans
Friedrich Miescher Institute for Biomedical Research, Basel.

To be or not to be a miRNA target site

Jean Hausser (M. Zavolan)
University of Basel, Biozentrum. 4056 Basel, Switzerland

MicroRNA feed back loop in cocaine induced synaptic plasticity and addiction

Vijay Chandrasekar (J.-L. Dreyer)
Institute of Biochemistry, University of Fribourg

Coffee break and poster session (16:05-17:00)

Quality control (17:00-17:40)

SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells

Pamela Nicholson (O. Mühlemann)
Institute of Cell Biology, University of Bern, Switzerland

Genome-wide analysis of the non-canonical poly(A) polymerases Trf4p and Trf5p: more than RNA quality control

Salvatore San Paolo (W. Keller)
Biozentrum, Cell Biology, University of Basel, Switzerland

Alternative coding (17:40-18:20)

In vivo analysis of selenocysteine synthesis shows a single pathway in eukaryotes

Eric Aeby (A. Schneider)
Department of Chemistry and Biochemistry, University of Bern

Alternative RNA editing creates novel proteins in trypanosome mitochondria

Torsten Ochsenreiter
Institute of Cell Biology, University of Bern

Analysis of pre-mRNA splicing in cell lines derived from patients with retinitis pigmentosa and dominant mutations in pre-mRNA splicing factor genes *PRPF31*, *PRPF8* and *PRPF3*

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Pre-mRNA splicing is a process in which introns are removed from pre-mRNA and exons are joined together to produce mRNA. It is catalyzed by complex molecular machinery termed spliceosome, composed of five snRNPs and numerous non-snRNP proteins. In addition to providing mature mRNA molecules to the cell, it contributes to protein diversity in higher eukaryotes through the process of alternative splicing, which allows the production of different mRNA molecules, and thus proteins, from a single pre-mRNA. Dysfunctions of splicing often lead to diseases and one of these is Retinitis pigmentosa (RP).

RP is the most common form of hereditary retinal degeneration, affecting 1/4000 individual worldwide. Clinically, it is characterized by progressive loss of vision and can lead to complete blindness because of the death of photoreceptors, the light-sensing cells of the retina. Genetically, this is a very heterogeneous disorder, caused by mutations in roughly 50 different genes. Most of these are retina-specific or have a well-defined role in the physiology of photoreceptors; others are expressed in many tissues or are ubiquitous and yet cause a phenotype that is retina-specific. Among the ubiquitous genes are *PAP-1* (RP9), *PRPF31* (RP11), *PRPF8* (RP13), and *PRPF3* (RP18), all encoding pre-mRNA splicing factors that are very conserved and essential for viability. All these genes encode proteins that are part of U4/U6-U5 tri-snRNP particle that represents, in addition to U1 and U2 snRNPs, a key component of the spliceosome.

The aim of our work is to understand why mutations in the ubiquitously expressed genes: *PRPF31*, *PRPF8* and *PRPF3* affect only one tissue – the retina. Moreover, from the mechanistic point of view, the nature of the mutations in these RP genes is different. Most of the *PRPF31* mutations lead to premature stop codons and degradation of the mutant mRNA by nonsense-mediated decay, therefore in heterozygous patients *PRPF31* is expressed at lower amounts and only from the wild-type allele, probably causing the disease through an haploinsufficiency-dependent mechanism. The RP-causing mutations in *PRPF8* and *PRPF3* genes are missense mutations or indels leading to premature stops in the last exon and may therefore potentially act as dominant-negative alleles. We are interested to know how decreased levels of *PRPF31* protein or mutations in *PRPF3* and *PRPF8* affect pre-mRNA splicing.

We have studied *in vitro* spliceosome formation, as well as *in vitro* and *in vivo* splicing using lymphoblastoid cells derived from RP patients carrying various mutations in the above-mentioned *PRPF* genes. Our results show that mutations in these genes slow down kinetics of spliceosome assembly; however they do not block splicing of the pre-mRNA tested. This could indirectly suggest that tissue-specific alternative splicing patterns could be affected in the case of *RP11*, *RP13* and *RP18* mutations and we are currently testing this hypothesis.

SR, hnRNP, PKC and EJC: Converging complexity to control the splicing of the apoptotic regulator Bcl-x

Benoit Chabot

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The Bcl-x pre-mRNA is alternatively spliced to produce the anti-apoptotic Bcl-xL and the pro-apoptotic Bcl-xS isoforms. We have identified a variety of exon elements that control the use of the 5' splice sites of Bcl-x. One element is bound by SRp30c to activate the xL site while others are bound by hnRNP F/H et hnRNP K to activate and repress the xS site. Another element strongly represses the xS site and requires protein kinase C activity. This sequence element appears to represent a converging platform for many signaling routes that monitor stresses and translation efficacy. Further, our screening platform has identified two components of the exon junction complex (EJC) involved in the control of Bcl-x splicing. These proteins also coregulate the alternative splicing of other apoptotic genes. Because EJC components are important for the decay of aberrant mRNAs with premature termination codons, a reduction in the level of these proteins may trigger apoptosis by encouraging the production of pro-apoptotic splice forms.

In vivo splicing modulation rescues a severe mouse model for Spinal Muscular Atrophy

Kathrin Meyer, Rachel Nlend Nlend, Judith Trüb, Julien Marquis and Daniel Schümperli, Institute of Cell Biology, University of Bern, Baltzerstrasse 4, CH-3012 Bern

Spinal muscular atrophy (SMA), the most common autosomal recessive disorder leading to infant death, is caused by homozygous deletion/inactivation of the *SMN1* gene (survival of motoneurons) and leads to the progressive degeneration of α -motoneurons in the spinal cord.

SMN1 codes for an ubiquitously expressed, essential protein with a major role in small nuclear ribonucleoprotein (snRNP) assembly. Survival of affected individuals into early childhood is due to a second gene (*SMN2*) that emerged from gene duplication. However, *SMN2* is no complete substitute, because a silent C-T transition impairs the inclusion of exon 7 in its mRNA. Most of the produced protein is truncated and unstable (SMN Δ 7). As all patients carry at least one *SMN2* copy, boosting its exon 7 inclusion is hypothesised to be an effective SMA therapy. So far, however, this has not been achieved *in vivo*, as it is difficult to specifically alter the splicing of a single mRNA with conventional drugs.

Our laboratory has pioneered methods to modulate the splicing of specific mRNAs with modified U7 snRNAs. In the case of *SMN2*, we found that a bifunctional U7 snRNA carrying an antisense sequence targeting exon 7 combined with a splicing enhancer sequence can efficiently stimulate exon 7 inclusion and SMN protein levels in SMA patient fibroblasts. Tests with an animal model lacking the mouse *smn* gene and carrying 2 copies of human *SMN2* show that the disease phenotype usually leading to death of these mice within 1 week can be overcome if they additionally contain the bifunctional U7 snRNA gene. While the oldest of these SMA/U7 mice are now nearly one year old, the median survival time depends on the integrated copy number of our therapeutic RNA and lies around 123 days. Besides being an important milestone towards the development of a somatic gene therapy for SMA, this result effectively proves the therapeutic value of an *SMN2* exon 7 inclusion strategy.

As it is still unclear if SMA is rather a developmental disorder, the time point when a therapy would have to be started is also unknown. To address this question we have generated a Doxocyclin inducible U7 that will be used for future transgenesis. Like that we will be able to start the splicing correction at different time points and will be able to evaluate if a treatment starting after birth will show equal benefits to the SMA mice.

NMR structure of the tra2 β 1 splicing factor in complex with RNA revealed an unexpected extensive interaction with the C-terminal extremity of the RRM

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Human transformer2-beta1 (tra2 β 1) protein is an SR-like protein that promotes in a concentration-dependent manner the inclusion of a large number of alternative exons [1, 2]. Despite its autoregulation, tra2 β 1 expression is unphysiologically high in breast and ovarian cancer [3, 4], hypoxia [5, 6], silicosis [7] and arteriosclerosis [8]. This protein contains two RS domains mainly involved in protein-protein interactions and a central RNA recognition motif (RRM) responsible for its specific interaction with RNA [1, 2]. Although the function of tra2 β 1 depends on its binding on pre-mRNA target sequences the molecular basis of this interaction is not well characterized.

Using NMR spectroscopy, we identified the AAGAAC sequence as the best candidate to study the structure of the tra2 β 1 RRM in complex with RNA. We solved the solution structure of this protein in complex with the 5'-AAGAAC-3' RNA. We obtained a precise structure due to 81 intermolecular NOEs. Interestingly, in addition to the β -sheet surface, the C-terminal extremity of the RRM is extensively used to bind the target RNA. In addition, our data clearly indicate that the GAA nucleotides are specifically recognized. The determination of this RNA-protein complex brings essential information on the specificity of RNA recognition by tra2 β 1 and then on its function and enriches the current knowledge about the versatile RRM-RNA interaction.

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2. Tacke, R., et al., Cell, 1998. **93**(1): p. 139-48.
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An assembly chaperone collaborates with the SMN-complex to generate spliceosomal snRNPs

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A structural hallmark of splicing U snRNPs is a ring shaped core that forms upon binding of Sm proteins onto U snRNA. PRMT5- and SMN-complexes are required, *in trans*, to mediate core assembly *in vivo* but their mode of function is unknown. We will report on the biochemical and structural dissection of core formation, which has yielded mechanistic insight into key steps of the assembly process. In an early phase of assembly, a ring-shaped subunit is formed on the PRMT5 complex composed of pICln and a distinct subset of Sm proteins. This stable pICln-Sm complex with a sedimentation coefficient of 6S is likely to be identical to an RNA-free assembly intermediate described by Blobel and co-workers decades ago, but whose function and composition has remained elusive. Sm proteins are incapable of assembling onto RNA out of this state due to a steric inhibition of the RNA binding site by pICln. The resulting kinetic trap is resolved by the SMN-complex, which contacts the 6S ring on the outer surface and ejects pICln. As a consequence, the formerly closed ring opens so that the Sm proteins can now contact the U snRNA. The SMN-complex then seals the ring around snRNA in a catalytic manner and releases the assembled U snRNP. Our data thus identify pICln as an assembly chaperone and the SMN-complex as a catalyst of the transition from pICln-Sm complexes to U snRNPs. Although disparate to DNA clamp loaders, this combined chaperone/enzyme system acts structurally similar.

Trans-acting antisense RNAs mediate transcriptional gene cosuppression in *S. cerevisiae*

Nissrine BEYROUTHY, F. Stutz lab, Department of Cell Biology, University of Geneva

Homology-dependent gene silencing, a phenomenon initially described as cosuppression in plants, depends on small interfering RNAs. We found evidence that in *S. cerevisiae*, which is missing the RNAi machinery, protein coding gene cosuppression exists. Indeed, transformation of a centromeric PHO84 plasmid or integration of an additional PHO84 gene within the genome result in the cosilencing of both the transgene and the endogenous gene. This repression is transcriptional, position independent and requires trans-acting antisense RNAs. We show that antisense RNAs induce transcriptional gene silencing both in cis and in trans and that the two pathways differ by the implication of the Hda1/2/3 HADC complex. Finally, we report that trans silencing is influenced by the Set1 histone methyltransferase, which regulates antisense RNA production. All together our data represent the first example of protein coding gene cosuppression in *S. cerevisiae* and highlight the role of antisense RNAs in mediating RNAi independent transcriptional gene silencing.

Yeast telomere repeat containing RNA (TERRA) and telomere length regulation

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Recent advances have demonstrated that surprisingly telomeres are actively transcribed into a non-coding telomere repeat containing RNA (TERRA), which remains associated with the telomere and may regulate telomere structure and maintenance (Azzalin et al, 2007; Schoeftner & Blasco, 2007). Interestingly, in human cells, the localization of TERRA to chromosome ends is under the control of the nonsense-mediated RNA decay (NMD) machinery. Our results demonstrate that in yeast TERRA levels are controlled by the conserved 5' to 3' exonuclease, Rat1, as inactivation of the temperature sensitive *rat1-1* allele results in rapid accumulation of TERRA. Through genetic approaches we have determined that the accumulation of TERRA in yeast, results in telomere shortening through the inhibition of telomerase. Strikingly TERRA induced telomere shortening can be overcome through the overproduction of RNaseH within the cells. These data support the idea that the formation of an RNA/DNA hybrid inhibits telomerase action at the chromosome end.

We have uncovered a link between the maintenance of telomere length homeostasis through Rap1 and the production of TERRA. Interestingly, cells expressing two different alleles of *RAP1*, *rap1-1* and *rap1-2*, have increased levels of TERRA while at the same time harbor short telomeres. The short telomere phenotype is not enhanced when the *rat1-1* mutation is introduced into either of these mutant backgrounds indicating an epistatic relationship. Taken together these data indicate that the achievement of telomere length homeostasis through the counting of Rap1 molecules may go through a non-coding RNA intermediate (TERRA) and that the number of Rap1 molecules may be influencing the rate of TERRA transcription.

Stress-dependent Coordination of Transcriptome and Translatome in Yeast

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Cells rapidly alter gene expression in response to environmental stimuli such as nutrients, hormones and drugs. During the imposed 'remodeling' of gene expression, changes in the levels of particular mRNAs do not necessarily correlate with those of the encoded proteins, which could in part rely on the differential recruitment of mRNAs to translating ribosomes. To systematically address this issue, we have established a novel approach to rapidly access the translational status of each mRNA in the yeast *Saccharomyces cerevisiae* by affinity-purification of endogenously formed ribosomes and the analysis of associated mRNAs with DNA microarrays. Using this method, we compared changes in total mRNA expression (transcriptome) with ribosome associations (translatome) after the application of different conditions of cellular stress. Severe stresses, induced by amino acid depletion or osmotic shock, stimulated highly correlated responses affecting about 15% of both transcriptome and translatome. Many of the regulated messages code for functionally related proteins, thus reflecting logical responses to the particular stress. In contrast, mild stress provoked by addition of Calcofluor-white and menadione altered the translatome of approximately 1% of messages with only marginal effects on total mRNA, implying largely uncorrelated responses of transcriptome and translatome. Among these putative translationally regulated messages were most components of the mitochondrial ATPase. Increased polysome associations of corresponding messages and higher mitochondrial ATPase activities upon treatment confirmed the relevance for regulation of this macromolecular complex. Our results suggest the presence of highly sensitive translational regulatory networks that coordinate functionally related messages. These networks are preferentially activated for rapid adaptation of cells to minor environmental perturbations.

Translational control by miRNAs and RNA-binding proteins

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Protein synthesis is controlled via two interdependent processes: the assembly of translation factors and ribosomes on the mRNA template, and the formation of mRNPs that promote or impede the former process. Hence, regulation of the activity of translation factors and modulation of mRNPs by regulatory trans-acting factors [RNA-binding proteins, microRNAs (miRs)] have emerged as the major principles for the control of protein synthesis.

Since the beginning, we have investigated the molecular mechanisms underlying translational control by trans-acting factors. Methodologically, the establishment of faithful cell-free systems has frequently served as a key step towards deciphering the underlying mechanisms. Specifically, I plan to discuss new insights into the regulation of *Drosophila* msl-2 mRNA translation by the RNA-binding protein Sex-lethal (SXL) and the co-repressor UNR, as well as the mechanism of translational control by *Drosophila* miR 2.

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Till et al., Nature Struct. Mol. Biol. 14, 897-903 (2007)

Duncan et al., Genes & Dev. 20, 368-379 (2006)

Beckmann et al., Cell 122, 529-540 (2005)

Function and Regulation of the *let-7* MicroRNA

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MicroRNAs (miRNAs) are a novel class of genes that account for >1% of genes in a typical animal genome. They constitute an important layer of gene regulation that affects diverse processes such as apoptosis, cell differentiation, and stem cell maintenance. Although it is generally agreed that miRNAs regulate their targets posttranscriptionally, through an antisense mechanism, the precise mechanism(s) of action remains controversial. Regulation at the level of mRNA stability, translation initiation, translation elongation, polyadenylation, and/or protein stability have all been invoked.

Guided by results from a genetic screen, where we established interaction between *C. elegans let-7* and the translation machinery, we used polysome profiling by sucrose density gradient centrifugation to demonstrate that *let-7* blocks translation of its endogenous targets at the initiation level. This finding overturns a dogma that miRNA repression occurs downstream of translation initiation *in vivo*, and we demonstrate repression of translation initiation for multiple, endogenous targets of multiple miRNAs. In addition, and reconciling some controversies in the field, we find that miRNA target transcript degradation frequently occurred together with translational repression, but appears to constitute a separate process. Finally, we find that the GW182 proteins AIN-1 and AIN-2 are essential both for miRNA target degradation and repression of translation initiation – an unexpected result as GW182 proteins were previously thought to play an auxiliary role, largely restricted to aiding mRNA degradation.

We have also begun to identify additional genes that are required for miRNA function, and we will discuss our progress on the characterization of these genes.

To be or not to be a miRNA target site

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How miRNAs recognize their target sites is a puzzle that many experimental and computational studies aimed to solve. Several features such as perfect pairing of the miRNA seed, additional pairing in the 3' region of the miRNA, relative position in the 3' UTR and the A/U content of the environment of the putative site have been found relevant.

Based on a large number of experiments comprising transcriptomics and proteomics datasets in addition to mRNA affinity profiles to the main effector of RNAi obtained through Ago2 immuno-precipitation, we have assessed the power that each of these features has in distinguishing between putative sites that do and those that do not appear to be functional. Our results indicate that the experimental datasets give widely different pictures of the importance of these features. They additionally suggest that miRNA target sites have been selected in evolution on their ability to have energetically favorable interactions with the miRNAs and to trigger mRNA degradation.

Finally, using the features we identified as most relevant for miRNA targeting, we trained a model to predict miRNA targets with improved accuracy. We show that moving away from the common "seed-based" approach to the prediction of miRNA-mRNA interactions, our model can predict sites that only match 5 of the first 8 nucleotides of the miRNA and yet induce mRNA degradation.

MicroRNA feed back loop in cocaine induced synaptic plasticity and addiction.

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Cocaine orchestrates synaptic plasticity by a surfeit of gene regulatory mechanisms. MicroRNAs, a class of small non-coding regulatory molecules, play a major role in temporal development and neurogenesis of complex physiological pathways in the brain. The focus of the present study is the elucidation of miRNA pathway in behavioral sensitization to cocaine and implications of differentially regulated miRNAs in the cocaine-induction of synaptic plasticity. Cocaine is one of the most reinforcing psycho-stimulant and blocks the dopamine transporter, thus inhibiting dopamine re-uptake and producing increased synaptic dopamine levels. Many axon guidance molecules implicated in cocaine mediated neural plasticity are regulated by miRNAs. A screening for cocaine specific miRNAs revealed the role of specific miRNAs. Quantitative RT-PCR analysis of several precursor and mature miRNAs showed that these miRNAs were significantly regulated under chronic cocaine administration in the mesolimbic dopaminergic system compared to saline treated controls. We found that mature miRNA profile correlates as expected with the pattern from precursor miRNAs in specific brain areas. These results were confirmed by the *miR-VANA* method (Ambion), by Northern blots and by *in-situ* hybridization analysis using LNA probes. Furthermore, miRNA- *in vitro* reporter assays using lentiviruses expressing miRNAs consolidated our observation of an inversely proportional concentration of targeting miRNAs and the targeted genes in cocaine addiction. Our results suggests, that miRNAs are involved in a complex signal transduction cascade activated by cocaine and may be involved in the regulation of many downstream genes upon drug treatment. The behavioral implication of expression of these miRNA genes in specific brain areas will be described.

SMG6 promotes endonucleolytic cleavage of nonsense mRNA in human cells

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In eukaryotes, mRNAs harbouring premature translation-termination codons (PTCs) are recognized and eliminated by nonsense-mediated mRNA decay (NMD). In addition to its quality control function, NMD constitutes a translation-dependent post-transcriptional pathway to regulate the expression levels of alternatively spliced mRNAs. Moreover, with an estimated 30% of all known human disease-associated mutations generating a nonsense mRNA, NMD is an important modulator of genetic disease phenotypes. Although NMD is conserved from yeast to man, previous reports suggest significantly different RNA degradation mechanisms between species. In *Drosophila*, an unidentified endonuclease initiates NMD by cleaving nonsense mRNA near the PTC, which exposes the two new ends for subsequent rapid exonucleolysis. In contrast, the current view posits that in yeast and human cells, elimination of PTC-containing RNA occurs from one or both termini, initiated by deadenylation and/or decapping followed by exonucleolytic decay. Here we report that also in human cells, degradation of nonsense mRNA can be initiated by a PTC-proximal endonucleolytic cleavage. Furthermore, we identify the metazoan-specific NMD factor SMG6 as the responsible endonuclease by demonstrating that mutation of conserved residues in the catalytic center of its nuclease domain - the C-terminal PIN motif - abolishes endonucleolysis *in vivo* and *in vitro*. Thus, our data reveal a new degradation pathway for nonsense mRNA in human cells, and suggest that endonucleolytic cleavage is a conserved feature in metazoan NMD.

Genome-wide analysis of the non-canonical poly(A) polymerases Trf4p and Trf5p: more than RNA quality control

Salvatore San Paolo, Walter Keller Lab, Biocenter, University of Basel

Trf4p and Trf5p are non-canonical yeast poly(A) polymerases that promote exosome-mediated degradation of aberrant and short lived RNA substrates. To assess the level of functional redundancy between Trf4p and Trf5p and to investigate the role of the Trf4-dependent polyadenylation *in vivo* we used DNA microarrays to compare the transcription profiles of the wild type (WT) strain of *S. cerevisiae* with either that of *trf4Δ* or *trf5Δ* mutant strains or the *trf4Δ* mutant expressing the polyadenylation defective Trf4(DADA) protein. We found that most of the transcripts that were upregulated in the *trf4Δ* or the *trf5Δ* mutants fell into two distinct groups of *trf4*-dependent and *trf5*-dependent transcripts. Surprisingly most RNAs, whose expression was affected by the *trf4* deletion, were restored to WT levels by the overexpression of *TRF4(DADA)* suggesting that the Trf4p-DADA is functional *in vivo*. Apart from previously reported RNAs, our analysis revealed that subsets of spliceosomal introns were also substrates for the Trf4 and Trf5 protein complexes (TRAMP4/TRAM5) and did not require the Trf4p-dependent polyadenylation for degradation. *In vivo* cross-linking and RNA immunoprecipitation-chip experiments (RIP-chip) provided further experimental support for a direct role of Trf4p in intron decay. In addition, although subtelomeric RNAs accumulated in the *trf4Δ*, the *trf5Δ* and the *rrp6Δ* mutants, only disruption of *trf4* caused a severe shortening of telomeres, suggesting that *TRF4* functions in telomere maintenance. Finally, we show that *TRF4*, the exosome and *TRF5* participate in antisense-RNA mediated regulatory pathways that modulate the expression of genes involved in phosphate metabolism.

In vivo analysis of selenocysteine synthesis shows a single pathway in eukaryotes

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Selenocysteine (Sec) is the 21st amino acid that is cotranslationally inserted into proteins of many organisms in response to UGA codons. A comprehensive in vivo study on the mechanism of selenoprotein synthesis in eukaryotes is still lacking. Here we have analyzed the in vivo Sec-tRNA^{Sec} formation pathway in the parasitic protozoa *Trypanosoma brucei*: selenoprotein synthesis was completely abolished in null mutants of either phosphoseryl-tRNA^{Sec} kinase or Sep-tRNA:Sec-tRNA synthase, demonstrating that there is a single pathway for Sec-tRNA^{Sec} formation requiring the sequential action of both enzymes. Growth of the two knock out strains was not impaired indicating that in trypanosomes, unlike in mammals, selenoproteins are dispensable for life. Moreover, analysis of conditional RNAi-strains indicates the essential roles of seryl-tRNA synthetase, selenophosphate synthase and Sec-specific elongation factor for selenoprotein synthesis. These results together with the recently in vitro reconstituted mammalian pathway strongly suggest that eukaryotes have a single highly conserved Sec-tRNA^{Sec} formation pathway.

Alternative RNA editing creates novel proteins in trypanosome mitochondria

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The mitochondrial genome of trypanosomes is composed of thousands of topologically interlocked circular DNA molecules that form the kinetoplast DNA (kDNA). Most genes encoded by the kDNA require a posttranscriptional modification process called RNA editing to form functional mRNAs. Here we show that alternative editing of the mitochondrial cytochrome c oxidase III (COXIII) mRNA in *Trypanosoma brucei* produces a novel DNA binding protein, alternatively edited protein-1 (AEP-1). AEP-1 localizes to the region of the cell between the kDNA and the flagellum and purifies with the tripartite attachment complex (TAC), a structure believed to physically link the kDNA and flagellar basal bodies. Expression of the DNA binding domain of AEP-1, results in aberrant kDNA structure and reduced cell growth indicating that AEP-1 is involved in the maintenance of the kDNA. Perhaps most important, our studies show a gain of function through an alternatively edited mRNA and for the first time, provide a link between the unusual structure of the kDNA and RNA editing in trypanosome mitochondria.

Poster no. 1.

Search of Pre-mRNA Splicing Alterations in Amyotrophic Lateral Sclerosis: a genomic-wide approach using Cellular Models.

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease caused by the degeneration of motor neurons. Although the cause of ALS is not known, mutations in the gene that produces the SOD1 enzyme are associated with some cases of familial ALS. SOD1 is a powerful antioxidant that protects the body from damage caused by superoxide, a toxic free radical. It has been proposed that defect in splicing of some mRNAs, can play a role in ALS pathogenesis. Alterations of splicing patterns have been also observed in ALS patients and in ALS murine models, suggesting that alterations in the splicing events can contribute to ALS progression. Using Exon 1.0 ST GeneChips, which allows the definition of alternative splicing events (ASEs), we have profiled SH-SY5Y neuroblastoma cell line upon treatment with a mitochondrial complex 1 inhibitor, which alters the patterns of alternative splicing. Furthermore, we have profiled the same cell line stably transfected with wild type and ALS mutant SOD. The integration of the two ALS models moderates efficiently ASEs false discovery rate which is one of the most critical issue in high-throughput ASEs detection. This approach allowed the identification of several splicing events affecting both internal coding exons and 5' UTR of known gene isoforms. Thus, a novel approach in the ALS research is presented, which permits us to accomplish a better understanding of the molecular mechanisms underlying ALS, this fundamental step is essential for the design of novel therapeutic strategies.

Poster no. 2.

Mutational Analysis of Mammalian SR Protein Kinase 2 (SRPK2)

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Tissue-specific alternative splicing profoundly affects animal physiology, development and disease, and this is nowhere more evident than in the nervous system. Alternative splicing is a versatile form of genetic control whereby a common precursor messenger RNA (pre-mRNA) is processed into multiple mRNA isoforms differing in their precise combination of exon sequences. In the nervous system, thousands of alternatively spliced mRNAs are translated into their protein counterparts where specific isoforms play roles in learning and memory, neuronal cell recognition, neurotransmission, ion channel function, and receptor specificity. The essential nature of this process is underscored by the finding that its misregulation is a common characteristic of human disease such as neurodegenerative disorders.

Our approach to gain insight into the regulation of neuron specific pre-mRNA splicing is to focus on the characterization of the kinase SRPK2. This protein phosphorylates serine/arginine-rich domain (RS-domain)-containing proteins and is expressed almost exclusively in the nervous system. SR proteins are a family of pre-mRNA splicing factors that play an essential role in constitutive and regulated splicing. The activity in pre-mRNA splicing and the subcellular localization of SR proteins are modulated by the level of phosphorylation of their RS domain.

In order to understand how SRPK2 intracellular localization and activity are regulated, we have constructed deletion mutants, as well as loss-of-function and gain-of-function mutants in Ser and Tyr residues that were shown to be phosphorylated. These mutants have been characterized by transient transfection in SH-SY5Y neuroblastoma cells and analyzed by immuno-fluorescence microscopy. Moreover, the mutants have been characterized under oxidative stress condition generated by pharmacological treatment of SH-SY5Y cells.

Poster no. 3.

Control of the DEAD-box RNA Helicase eIF4A by eIF4G

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Translation initiation in eukarya is usually the rate-limiting and most tightly controlled stage of polypeptide synthesis. A set of about a dozen factors (eIFs) ensures efficiency and fidelity of this process (Marintchev A et al. (2004) *Q Rev Biophys* 37: 197-284). A central component is eIF4F, a complex consisting of the cap-binding protein eIF4E, the ATP-dependent DEAD-box helicase eIF4A and a scaffold protein eIF4G. The structure of the complex of eIF4A and middle domain of eIF4G has been solved recently (Schuetz P et al. (2008) *PNAS* 105: 9564-9569). eIF4A shows an extended conformation where eIF4G holds its crucial DEAD-box sequence motifs in a productive conformation, thus explaining the stimulation of eIF4A's RNA helicase activity. Conformational changes between eIF4A's closed and open state provide a model for RNA-helicase activity and its enhancement by the interaction with eIF4G. In the open state there are three main interaction sites, one of them involves a flexible element in the N-terminal region of eIF4G's middle domain. The key residue in this site is Trp-579, where mutation to alanine decreases eIF4G's binding affinity for eIF4A significantly. Owing to its pocket shape and small surface, this interaction site might be a good target for the development of anticancer drugs. mRNAs containing long, structured 5'-UTRs, which are also present in several proto-oncogenes and somatic growth factors, have a high requirement for eIF4F. A possible strategy for anticancer drugs is therefore the development or identification of small-molecule compounds that are able to disrupt the eIF4F complex (Kim WJ et al. (2007) *EMBO J.* 26: 5020-32).

Poster no. 4.

Characterization of NONO – a RNA-binding Protein with pleiotropic Functions

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We have identified the NONO protein as a member of a circadian clock protein complex that also contained Period and Cryptochrome proteins. Depletion of this protein in mammalian cells or mutation of its homologue in flies resulted in severe attenuation of circadian rhythmicity, possibly due to antagonism of Period-mediated transcriptional repression (Brown *et al.*, *Science* 2005). Previously, this RNA-binding protein has also been implicated in diverse processes of transcriptional regulation, as well as in RNA nuclear export, transport, and metabolism.

To better understand NONO function both within and outside the circadian oscillator, we have employed a “gene-trapped” NONO male murine embryonic stem (ES) cell line that completely abrogates the production of transcripts containing coding sequences for this X-linked gene. Although ES cells divide rapidly and possess no detectable circadian transcriptional oscillations, wildtype cells possess normal circadian rhythmicity once differentiated into neuronal lineages. By contrast, complete loss of rhythmicity is observed in comparably-differentiated NONO-genetrap cells. By standard blastocyst injection, we have generated NONO-genetrap mouse lines whose behavioural characterization will be presented. Results with these mice show a slightly shortened period of the circadian oscillator when compared to their wildtype littermates in free-running (dark/dark) conditions.

To explain the discrepancy between the phenotypes observed *in vitro* and *in vivo* we searched for homologues that could substitute for NONO function during development. The two characteristic RNA recognition motifs (RRMs) followed by a specific DNA-binding domain were found in 4 other proteins via conserved domain architecture. *In vitro* assays show comparable circadian phenotypes in these homologues, suggesting a new family of proteins implicated in the circadian clock. Using deletion and overexpression constructs we currently are examining the individual functions of NONO and its homologues as well as the contribution of the different domains.

Poster no. 5.

Elucidating the mechanism of GLD-1 mediated repression in the *C.elegans* germ line

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Translational regulation is widespread in germ cell development, including in *C. elegans*. Various translational regulators are expressed in distinct regions of the *C. elegans* germ line. These factors usually control the temporal and spatial expression of their mRNA targets by binding to regulatory elements in the 3'-UTR. Many of these regulators have been shown to be present in germline RNP granules. However the significance of this association is not clear.

The STAR (signal transduction and RNA) domain protein GLD-1 is a key translational regulator in the *C. elegans* germ line, and is known to repress a number of mRNAs in the central region of the gonad. The mechanism by which GLD-1 represses translation of mRNAs however is largely unknown.

To investigate the mechanism of GLD-1 mediated translational regulation we analyzed the association of GLD-1 regulated transcripts with ribosomes. We found that the association with ribosomes varies among GLD-1 targets, indicating that additional co-factors influence how these mRNAs are regulated. Indeed, we observed that GLD-1 interacts with components of P body-related germline granules, called storage granules. Interestingly, the stability of at least one GLD-1 target mRNA requires storage granules, suggesting a connection between GLD-1-dependent repression and storage granule-mediated mRNA stability. This connection will be further examined in future experiments.

Poster no. 6.

A minimal RNA sequence requirement for GLD-1 mediated translational repression *in vivo*.

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The *C.elegans* gonad contains germ cells in a linear sequence of development. Many of the key decisions in germline development such as the mitosis to meiosis transition and the switch between spermatogenesis and oogenesis involve translational regulation mediated by the GLD-1 (defective in Germ Line Development) protein. GLD-1 is a KH domain RNA-binding protein of the STAR family and ortholog of mammalian Quaking and *Drosophila* HOW. Loss of GLD-1 leads to a germ cell tumor and transdifferentiation into somatic cells [1, 2]. GLD-1 regulates several mRNAs in the gonad including the notch receptor *glp-1*, *cep-1* (p53), *cye-1* (cyclin E), *pal-1* (caudal) and that of the yolk receptor protein RME-2. *In vitro* analysis has identified putative GLD-1 binding elements [3]. However it is not known if these binding elements are important for GLD-1 mediated translational repression *in vivo*. We find that the *rme-2* and *cye-1* 3'UTRs are sufficient to impose GLD-1 mediated repression *in vivo*. Furthermore, mutation of putative GLD-1 binding elements within the *rme-2* and *cye-1* 3'UTRs alleviates GLD-1 binding *in vitro* and translational repression *in vivo*. In addition, we have identified GLD-1 mRNA targets by GLD-1 IP, followed by microarray analysis of associated mRNAs. Interestingly, we find significant enrichment of the putative GLD-1 binding elements within the 3'UTRs of GLD-1 associated mRNAs. Remarkably, introduction of a single GLD-1 binding element into an unrelated 3'UTR is sufficient to confer GLD-1 mediated translational repression *in vivo*. Our future objective is to understand the precise mechanism by which GLD-1 acts as a translational repressor in the *C.elegans* germ line.

1. Francis, R., et al., *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. Genetics, 1995. **139**(2): p. 579-606.
2. Ciosk, R., M. DePalma, and J.R. Priess, *Translational regulators maintain totipotency in the Caenorhabditis elegans germline*. Science (New York, N.Y., 2006. **311**(5762): p. 851-3.
3. Ryder, S.P., et al., *RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1*. Nat Struct Mol Biol, 2004. **11**(1): p. 20-8.

Poster no. 7.

Cotranslational assembly of the yeast SET1C histone methyltransferase complex

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Although most eukaryotic proteins are assembled in multi-protein complexes little is known about how these complexes are formed. The *Saccharomyces cerevisiae* SET1C/COMPASS histone methyltransferase is a nuclear multi-protein complex comprising eight different polypeptides including Set1, the enzymatic subunit. We found evidence that the initial event in SET1C formation is the establishment of a SET1 mRNA associated complex (SET1RC) harbouring SET1 mRNA as well as four of the eight proteins forming SET1C. Our data suggest that SET1RC assembles on nascent Set1 in a translation dependent manner. Furthermore, SET1RC is a transitional complex that requires key complex components, including the WD repeat proteins Swd1, Swd2 and Swd3 for further maturation into SET1C. We propose that cotranslational assembly of SET1RC is part of a quality control system monitoring the fidelity of complex formation thereby protecting the cell from the potentially deleterious effects of an incomplete or miss-assembled methyltransferase. Consistent with this idea the catalytic Set1 subunit is targeted for rapid degradation when Swd1/2/3 proteins are not available at the required stoichiometry. Our working model is that cotranslational complex assembly and cotranslational protein degradation act in kinetic competition. Preliminary results suggest the involvement of proteasomal degradation of Set1 when Swd proteins are underrepresented. We are currently testing whether this involves cotranslational ubiquitylation to target Set1p for degradation. The earliest and therefore most effective quality control mechanism for complex assembly is a cotranslational one. Therefore, we consider it likely that other protein-complexes are formed and controlled in a manner similar to SET1C.

Poster no. 8.

Analyzing the role of yClp1 and its ATP binding activity in pre-mRNA 3' end formation in *Saccharomyces cerevisiae*

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Pre-mRNA 3' end formation is a two-step reaction of endonucleolytic RNA cleavage followed by poly(A) addition to the upstream cleavage product. The characterization of the machineries catalyzing these reactions in yeast and metazoan cells identified large multi-protein complexes. In yeast, cleavage factor IA (CF IA) together with cleavage and polyadenylation factor (CPF) are sufficient to catalyze cleavage and polyadenylation *in vitro*. CF IA is composed of four subunits: the RNA polymerase II CTD binding protein Pcf11, the RNA binding protein Rna15, Rna14 and yClp1. Interestingly, yClp1 carries an evolutionary conserved P-loop motif characteristic of ATP binding proteins. While ATP binding activity has been demonstrated for yClp1 (1) its functional role during pre-mRNA 3' end formation remains unclear. An exciting recent finding was that human hClp1 displays 5' RNA kinase activity required for pre-tRNA splicing (2). However, such an activity has not been found associated with yClp1 (3). We are analyzing the role that yClp1 and in particular its ATP binding activity play during pre-mRNA and snoRNA 3' end formation and RNA polymerase II transcription termination. To this end we analyzed the effects of *in vivo* depletion of yClp1 by promoter shut-off and observed defective 3' end formation at *ACT1* and *ASCI* 3' ends and transcriptional read-through at the *CYH2* terminator. These observations establish a requirement for Clp1 in pre-mRNA 3' end formation *in vivo*. Currently, we are analyzing the effects that mutations of the ATP binding site within Clp1 have on both 3' end formation and RNA polymerase II transcription termination. In addition, we are characterizing a physical interaction that we previously detected between Clp1 with Ysh1, the putative 3' endonuclease associated with CPF.

1) Noble et al., *NAR*, 2007; 35(1): 87-99

2) Weitzer and Martinez, *Nature*, 2007; 447(7141): 222-6

3) Ramirez et al., *RNA*, 2008; 14(9): 1737-45

Poster no. 9.

Systematic analysis of the crosstalk between regulatory RNA-binding proteins and the microRNA machinery

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The important role of post-transcriptional events in the regulation of gene expression is increasingly being recognized. In recent years, various examples of such post-transcriptional gene regulation have been described, mostly relying on RNA-binding proteins (RBPs) and (small) non-coding RNAs such as microRNAs. Reminiscent of transcription factors in transcriptional regulation, these molecules form large dynamic protein complexes (“mRNPs”) that bind *cis*-acting elements often present in subsets of mRNAs that are functionally related. Depending on the exact composition, i.e. the presence or absence of *cis*- and *trans*-acting and accessory factors, mRNP formation in the cytoplasm generally alters message localization, stability or translation rate. However, in contrast to transcriptional regulation, details and mechanisms of post-transcriptional regulatory systems, and especially their interplay, is still poorly understood.

We wish to address these questions by elucidating post-transcriptional regulatory networks. To achieve this, we have established an immunopurification method that allows us to identify RNA and proteins components of mRNPs in human cells, using a ribonomics approach and mass spectrometry, respectively. In particular, we are interested to identify RBPs that crosstalk with the microRNA machinery, as well as their putative “co-targets”. Bioinformatics, biochemical and functional analyses will then be applied to gain insight into the nature and extent of post-transcriptional gene regulation in the cytoplasm. Based on previous studies performed in our group, we will initially focus on the role of PUM1 in microRNA regulation, as suggested by bioinformatics analyses and preliminary experimental results.

Poster no. 10.

Global Identification of Potential RNA Targets for the Paralogous La-related RNA binding Proteins Slf1p and Sro9p

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In eukaryotic cells, regulation of translation plays important roles in controlling gene expression and is exerted both on a global scale and on specific mRNAs. Control of individual transcripts is often mediated by specialized RNA-binding proteins (RBPs) that recognize sequence/structural elements in the untranslated regions (UTRs) of mRNAs. However, to date, the RNA targets of the numerous known or predicted eukaryotic RBPs are largely unknown. By means of DNA microarrays we aim at determining the RNA targets of yeast RBPs that associate with ribosomes and are thus prime candidates to specifically regulate mRNA translation.

Our investigations have focused on two evolutionary conserved La-motif (LM) containing proteins from the yeast *Saccharomyces cerevisiae*, termed Slf1p and Sro9p. These proteins have previously been shown to bind RNA homopolymers *in vitro* and to preferentially associate with polysomes (Sobel & Wolin, 1999). We found that the two proteins bind to a largely overlapping set of hundreds of mRNAs. In contrast to the bona fide La protein, which binds to the 3' polyuridine tail of nascent RNA polymerase III transcripts, we could not find significant association of noncoding RNAs with Sro9p and Slf1p. This is in agreement with the fact that Slf1p and Sro9p are mainly cytoplasmic whereas La proteins are primarily localized in the nucleus. In addition, La related proteins do not contain a RNA recognition motif (RRM), which is proposed to act in concert with the La domain and consequently confer the specificity of polyuridine-tail recognition to La proteins. Among the Slf1 and Sro9 RNA targets are most transcripts coding for components of the cytoplasmic ribosome, the histones as well as many mRNAs of genes involved in secretion. We also find transcripts that are involved in copper homeostasis which may link to previous findings that implicate Slf1p in copper detoxification. Indeed, we could confirm that overexpression of *SLF1* leads to enhanced growth in elevated copper concentrations, whereas deletion of the gene shows opposite effects. However, similar experiments with *SRO9* didn't suggest a role for Sro9p in copper detoxification.

In current experiments we aim at monitoring the effects of overexpression and deletion of Slf1p on target mRNA levels. Preliminary data obtained by Microarray analysis and qRT-PCR suggest that Slf1p exerts a stabilizing effect on its mRNA targets.

Poster no. 11.

Systematic identification of RNA-binding proteins in yeast reveals Gis2p as a global post-transcriptional regulator and potential model for the cause of human myotonic dystrophy

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RNA-binding proteins (RBPs) are implicated in diverse aspects of post-transcriptional gene regulation. Many RBPs contain characteristic RNA-binding motifs which allowed prediction of hundreds of RBPs in eukaryotic genomes using bioinformatic tools. To identify novel RBPs, which might contain thus far unknown RNA-binding motifs, we have established a procedure to systematically detect RNA-interacting proteins: By applying fluorescently labeled RNA on high-density protein microarrays, which contain more than 4000 proteins from the yeast *Saccharomyces cerevisiae*, we identified more than 150 proteins that reproducibly interacted with mRNA. Surprisingly, many of them are proteins with previously known functions, e.g. metabolic enzymes.

For 14 of these potential RBPs, we performed affinity isolations of tagged proteins from yeast cell extracts. The associated RNAs were analyzed with DNA microarrays to identify cellular RNA targets. We found that each of these RBPs was associated with a unique set of RNAs, many of them coding for functionally related proteins.

One of these RBPs, Gis2p, bound more than 1000 mRNAs covering 1/6 of all yeast genes. With RNA pull-downs we found that Gis2p binds to its target mRNAs preferentially through interaction with the open reading frame region, and using bioinformatics we could determine a repetitive GAN pattern responsible for the binding. Likewise, the human Gis2 homolog Znf9, a protein whose mutation causes myotonic dystrophy type 2, shows similar mRNA binding specificities. Therefore, coordinated post-transcriptional regulation of a large number of proteins by Znf9 could be an explanation for the severe phenotype observed in myotonic dystrophy patients.

Poster no. 12.

Mechanisms involved in efficient removal of multiple introns

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The vast majority of mammalian pre-mRNAs contains multiple introns that are excised prior to export and translation. The order of intron removal varies from pre-mRNA to pre-mRNA and can occur sequentially in a strict 5' to 3' direction, or independent of the linear order of intron appearance. After intron excision, ligated exon intermediates participate in subsequent intron excisions. However, exon ligation generates an exon of increased size, a feature of pre-mRNA splicing that can potentially interfere with downstream splicing events. Previous studies have demonstrated that large mammalian exons are poorly recognized by components of the spliceosome and most mammalian exons are confined to an average size of 150 nts. This reasoning argues that spliceosome assembly on ligated exons may limit the efficiency of additional intron excisions. However, recent experiments have also shown that mammalian splice sites are recognized co-transcriptionally. Thus, it is possible that ligated exon intermediates do not have to go through exon definition again, thereby circumventing the potential exon size restrictions. These considerations raise the question whether unique mechanisms exist that permit efficient removal of introns neighboring ligated exons, a proposal supported by preliminary data. Results from in vitro and cell transfection approaches will be discussed testing the hypotheses that (1) the recruitment of the EJC to ligated exons increases the efficiency of multiple intron excisions or that (2) the initial definition of splice sites is sufficient to permit efficient splicing of introns neighboring ligated exons.

Poster no. 13.

A conformational change in the helicase core is necessary but not sufficient for RNA unwinding by the DEAD box helicase YxiN

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The helicase core of DEAD box helicases contains the conserved helicase motifs for ATP binding and hydrolysis, RNA binding, and coupling of ATP hydrolysis to RNA unwinding. ATP is bound in the cleft between the two RecA domains, and RNA interacts with a bipartite binding site formed by both domains. Cooperative binding of ATP and RNA substrate induces the formation of a closed conformer with an extensive network of interactions between conserved motifs across the domain interface. The RNA bound to the closed conformer is bent, and the introduction of this kink may be a first step towards RNA unwinding. While few mutations have been identified that uncouple ATP hydrolysis from RNA unwinding, the coupling mechanism is not well-understood. To dissect the role of the conformational change in the helicase core for RNA unwinding, we characterized the RNA-stimulated ATPase activity, RNA unwinding, and the propensity to form the closed conformer for an ATPase-deficient and two “uncoupled” mutants of the DEAD box helicase YxiN. The hydrolysis-deficient K52Q mutant forms a closed conformer upon binding of ATP and RNA, but is deficient in RNA unwinding. A mutation in motif III slows down ATP hydrolysis and thus the overall catalytic cycle, but neither affects the propensity for the closed conformer nor its global conformation. In contrast, the G303A mutation in motif V prevents a complete closure of the inter-domain cleft, affecting ATP binding and hydrolysis as well as RNA binding, and is detrimental to unwinding. We propose that the K52Q and motif III mutants still introduce a kink into the backbone of bound RNA, whereas G303A fails to kink the RNA substrate. Consequently, the closure of the cleft in the helicase core is necessary but not sufficient for RNA unwinding by DEAD box helicases.

Poster no. 14.

Biogenesis and Degradation of Telomeric Repeat Containing RNA (TERRA) in the Yeast *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, the Rat1 5' to 3' exonuclease is involved in efficient transcription termination. The rat1-1 thermosensitive mutant accumulates at the restrictive temperature high levels of RNA polymerase II transcripts containing telomeric repeats (TERRA) and displays a marked telomere shortening phenotype when grown at semi-permissive temperature. We will present data which demonstrate that TERRA accumulation in rat1-1 mutants arises from inefficient transcription termination of the most external ORFs at chromosome ends. However, we employed telomere-specific RT-PCR to detect TERRA transcripts from Y' prime and not-Y'prime telomeres. This revealed the presence of low, but clearly detectable TERRA also in wild type cells. Furthermore we found that TERRA 3' ends are polyadenylated and that Rat1p also destabilizes TERRA molecules that are derived from Gal1-promoter driven expression of a telomere. Thus, we entertain the idea that TERRA is generated not only through inefficient transcription termination from upstream ORFs but also from transcripts initiated in the immediate vicinity of telomeric repeats. Experiments are underway to further characterize the mechanisms, which contribute to TERRA biosynthesis.

Poster no. 15.

Mucosal Proteases and their Inhibitors in Inflammatory Bowel Disease

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The IBDase project is a European research consortium addressed to the etiology and pathogenesis of inflammatory bowel disease (IBD), focused on mucosal proteases and their inhibitors (P/PIs). To this end we study human mucosal P/PI polymorphisms affecting expression levels and/or activity of P/PIs and analyze their genotype/phenotype associations in IBD patient cohorts across Europe. The multidisciplinary effort of the consortium aims to identify molecular mechanisms for involvement of IBD specific P/PI gene variants and to innovate diagnosis and therapy.

The initial work package was dedicated to identify P/PIs with highest impact on susceptibility and pathogenic mechanisms in IBD. The lists of potentially relevant P/PIs in IBD were generated on the basis of a systematic review of genetic and expression studies. These results will now become subject of genetic and functional studies to be followed up in the future work packages. One of the functional studies work packages is dedicated to get insights of the expression and function of P/PIs in the IBD pathogenic mechanisms, *in vitro*. We are planning to investigate the differential role of miRNAs in the expression regulation of P/PI variants that contain polymorphisms within predicted miRNA target sites, for instance miRNA-559 in mepirin-alpha. IBD-associated P/PI variant sequences (haplotypes) will be incorporated into luciferase-based reporter systems using site-directed mutagenesis and analyzed in cells co-transfected with reporter system and corresponding miRNA's. Specificity of the effects will be determined by comparison of miRNA-deficient (Dicer -/-) and control cell lines.

These results integrated with the others will lead to the detailed knowledge on the role of P/PI genes influencing the aberrant function of mucosal P/PIs modulating IBD susceptibility and causing dysfunctional pathways. That information will be used to: propose specific P/PIs to be targeted in IBD treatment, deduce novel therapeutic targets from the identified pathways, and improve accurate diagnosis in IBD.

Poster no. 16.

Role of microRNAs in regulation of the tachykinin receptor gene expression in patients with interstitial cystitis

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Interstitial cystitis (IC) is a clinical syndrome of pelvic pain and/or urinary urgency/frequency in the absence of a specific cause such as bacterial infection or bladder injury. The pathogenic mechanisms are as yet undefined, with epithelial dysfunction, mast cells activation, and neurogenic inflammation as possible causative mechanisms. Substance P and related tachykinins mediate smooth muscle contraction, vasodilation, and inflammation through stimulation of neurokinin 1 (NK1) and neurokinin 2 (NK2) receptors. To uncover the link between the clinical symptoms and molecular regulation of the atypical inflammatory response, we studied the expression levels of tachykinin receptors NK1 and NK2 in IC patients, using quantitative real-time PCR. Bladder biopsies were collected from 8 controls, and 16 IC patients with high degree of pain.

The results show a significant ($p < 0.05$) down-regulation of the RNA levels of NK1 and NK2 receptors in the bladder of IC patients compared to the controls. In contrast, purinergic receptor P₂X₁, involved in the regulation of smooth muscle contraction, did not show any differences between the examined groups. Our data indicate the involvement of the neurokinin receptors of the bladder dome in the molecular pathogenesis of IC.

In an effort to understand the mechanisms of down-regulation of the tachykinin receptor expression, we investigated the role of microRNAs in IC. MicroRNAs are non-coding single-stranded RNA molecules of *ca.*22 nucleotides, which regulate the mRNA translation, either by blocking the mRNA translation or promoting mRNA degradation. They have been implicated in the pathogenesis of several chronic inflammatory disorders, including psoriasis, inflammatory bowel disease and respiratory inflammation.

We used Taqman Low Density Microarrays (Applied Biosystems) to screen for the presence of 348 microRNAs in the samples isolated from 8 IC patients' biopsies and 4 control biopsies. Among microRNA, significantly up-regulated ($p < 0.05$) in IC patients, five have tachykinin receptors NK1R and NK2R as predicted target genes: miR-192, -199a, -320 and -328 for NK1R and miR-95 for NK2. We tested these miRNAs for their ability to inhibit NK1R expression in a cell-based assay. Transfecting the human astrocytoma U373MG cell line with Pre-miR-328 resulted in a significant down-regulation ($p < 0.05$) of NK1R mRNA levels, implicating this miRNA in regulation of NK1R expression during IC.

Poster no. 17.

Processing bodies are not required for mammalian nonsense-mediated mRNA decay

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic quality-control mechanism that recognizes and degrades mRNAs with premature termination codons (PTCs). In yeast, PTC-containing mRNAs are targeted to processing bodies (P-bodies), and yeast strains expressing an ATPase defective Upf1p mutant accumulate P-bodies. Here we show that in human cells, an ATPase-deficient UPF1 mutant and a fraction of UPF2 and UPF3b accumulate in cytoplasmic foci that co-localize with P-bodies. Depletion of the P-body component Ge-1, which prevents formation of microscopically detectable P-bodies, also impairs the localization of mutant UPF1, UPF2 and UPF3b in cytoplasmic foci. However, the accumulation of the ATPase-deficient UPF1 mutant in P-bodies is independent of UPF2 or UPF3b. Most importantly, disruption of P-bodies by depletion of Ge-1 affects neither the mRNA levels of PTC-containing reporter genes nor endogenous NMD substrates. Collectively, our results demonstrate that P-bodies are not required for mammalian NMD.

Poster no. 18.

The RNA Helicase RHAU is not only involved in mRNA metabolism but also in chromatin modification.

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RHAU, a putative DExH box RNA helicase, has been shown to accelerate the ARE-dependent mRNA degradation of uPA-mRNA. Other components of this decay machinery are NF90, PARN and the exosome and more recently found: Upf1, Upf2 and SMG1 which are also components of the nonsense mediated decay mechanism. Additionally RHAU is able to localize to stress granules upon arsenite treatment with its N-terminal unique RNA-binding domain responsible for this feature.

By YTH we found PHF1b as a new interaction partner of RHAU. PHF1b is a component of a subclass of nuclear PRC2 complexes which are responsible for the trimethylation of Histone 3 at Lysine 27 which cause HOX Gene Repression and inactivation of the X Chromosome. By co-immunoprecipitation experiments we could confirm a weak interaction between RHAU and PHF1b and additionally a surprisingly even stronger interaction of RHAU with Ezh2, the Histone methyltransferase of PRC2. Examinations of the methylation status of H3K27 in HeLa cells upon RHAU KD by siRNA showed an increased trimethylation in parallel with an reduced mono- and di-methylation of H3K27. Same results we obtained in MEF cells in which RHAU is completely knocked out. Thus RHAU seems to negatively influence the activity of Ezh2. Since for PHF1b the opposite effect is reported, both proteins, RHAU and PHF1 are maybe antagonists in the same mechanism. Further studies have to clarify more in detail how RHAU exactly influence Histone methylation and which target genes are affected.

Poster no. 19.

The ubiquitin-related modifier Urm1 acts as a sulfur-carrier in thiolation of eukaryotic tRNA

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Ubiquitin-like proteins (UBLs) can change protein function, localization or turnover by covalent attachment to lysine residues. While UBLs achieve this conjugation via an intricate enzymatic cascade, their bacterial counterparts MoeA and ThiS function as sulfur-carrier proteins. We identified the *URM1* pathway as required for the thiolation of three cytoplasmic tRNA species in yeast by systematic genetic analysis (SGA). Furthermore, we showed that Urm1p, the most ancient UBL, acts as a sulfur-carrier in this process independent of its function as a protein modifier. Thus, Urm1p might provide an evolutionary link between UBL and sulfur transfer.

Moreover, we identified Uba4p, Ncs2p, Ncs6p and Yor251cp as components of this pathway. We could show that Ncs2p and Ncs6p interact and that Ncs6p can bind tRNA *in vitro*. Furthermore, we show that Uba4p first adenylates and then directly transfers sulfur onto Urm1p. Using *Caenorhabditis elegans* and human cells we established that the pathway is conserved in higher eukaryotes, leading to interesting medical perspectives. Finally, functional analysis revealed that the thiolation function of Urm1p is critical to regulate cellular responses to nutrient starvation and oxidative stress conditions, most likely by increasing translation fidelity.

Poster no. 20.

Conditional vitamin-mediated repression of chloroplast gene expression in *C. reinhardtii*

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Over the last decades, the green alga *Chlamydomonas reinhardtii* has emerged as a powerful model system for studying the chloroplast molecular biology. Yet, our knowledge of the function of essential chloroplast genes remains very limited. In order to address this issue, the development of a novel repressible chloroplast gene expression system is currently underway. At the molecular level, the system is based on a genetically engineered version of the nucleus-encoded chloroplast Nac2 protein which is specifically required for stabilization of the endogenous *psbD* mRNA encoding the D2 reaction center polypeptide of PSII (Nickelsen J. et al., Plant Cell 1999; Surzycki R. et al., PNAS 2007). Expression of *Chlamydomonas* genes can be tightly controlled by their artificial fusion to a B12-responsive promoter and a TPP-riboswitch within the 5'UTR (Croft MT. et al., Nature 2005; Croft MT. et al., PNAS 2007). Thus, when the *nac2* mutant strain is transformed with a chimeric Nac2 gene driven by the vitamin-responsive promoter and 5'UTR, a conditional repression of D2 synthesis and, in turn, loss of PSII activity, is indeed observed when B12 and TPP are added to the growth medium. Ongoing studies are therefore focused on testing whether the Nac2 vitamin-responsive system can also be used to achieve conditional knock-down of essential plastid genes, by fusion of the Nac2 target site, the *psbD* 5'UTR, to any other chloroplast ORF, in a photoautotrophic strain where the endogenous *psbD* 5'UTR has been replaced with another chloroplast 5'UTR. At the same time, we are also planning to exploit the system for other biotech applications, like transient expression of foreign proteins of commercial interest and improvement of hydrogen bio-production.

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Poster no. 21.

Functional characterisation of 3' processing factors *in vivo*

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Post-transcriptional cleavage of pre-mRNA is an essential step in mRNA maturation. Following cleavage, most eucaryotic mRNAs, with the exception of replication-dependent histone transcripts in metazoa, acquire a poly(A) tract at their 3' ends. The mature 3' ends of the metazoan replication-dependent histone mRNAs, however, lack a poly(A) tail and are generated by a single endonucleolytic cut. In both cases, the process of 3'-end formation promotes transcription termination and transport of the mRNA from the nucleus.

The most obvious difference between the two processing mechanisms is the requirement for an RNA containing factor (the U7 snRNP) in histone pre-mRNA processing and the lack of an RNA component in cleavage/polyadenylation. Even though the generated 3' ends are different, several factors are shared between the two processing reactions. Among them the endonuclease, the 73 kDa subunit of cleavage and polyadenylation specificity factor (CPSF-73), which strongly suggests that the two 3' end processing mechanisms evolved from a common primordial machinery.

Since most data derive from *in vitro* studies, we decided to study the 3' processing reactions *in vivo* to investigate the functional role of the factors that are shared between the different processing reactions. To do so, we depleted cells from several factors by RNA interference and studied the effect on the processing efficiency by quantitative RT-PCR. Indeed, we can show that CPSF-73 is the 3' endonuclease *in vivo* for both types of mRNAs, however the *in vivo* approach raises new questions regarding the 3' processing of polyadenylated mRNAs.

Poster no. 22.

The nuclear pore complex regulates *GAL1* activation by controlling the sumoylation of transcription repressors

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Transcription activation of some yeast genes is paralleled by their repositioning to the NPC. This mechanism requires NPC constituents, transcription factors and components of the mRNA-processing and export machinery. Mlp proteins have been involved in this process and they are also known to restrict the substrate specificity of Ulp1, a key sumo-protease, by maintaining this enzyme at the NPC. In strains lacking Mlp proteins, Ulp1 is released from the NPC. Interestingly, the kinetics of *GAL* gene activation are increased in this strain. We propose that Mlp proteins, through the action of Ulp1, participate in transcription regulation at the nuclear periphery by desumoylating transcription factors. Tup1 and Ssn6 are general transcription repressors involved in the repression of *GAL* genes by glucose. We identified a two-hybrid interaction between Tup1/Ssn6 and the NPC-associated Mlp2 protein. Notably, Tup1 is sumoylated and our data indicate that its sumoylation state is regulated by Ulp1. We propose that Mlp proteins, through the action of Ulp1, participate in transcription regulation at the nuclear periphery by desumoylating Tup1 and Ssn6.

Poster no. 23.

Ubiquitination of Yra1 by Tom1 regulates its interaction with Nab2 and Mex67 during yeast mRNA export

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The evolutionarily conserved mRNA export receptor Mex67/NXF1 is critical for gene expression. Mex67 associates with mRNAs through its adaptor, Yra1/REF, allowing their exit through nuclear pores. However, alternate adaptors should exist since Yra1 is dispensable for mRNA export in *Drosophila* and *C. elegans*. Here we report that Mex67 directly interacts with Nab2, an essential shuttling RNA-binding protein required for mRNA polyadenylation and export. We further show that Yra1 promotes the interaction between Nab2 and Mex67 and becomes dispensable in cells over-expressing Nab2 or Mex67. Notably, ubiquitination of Yra1 by the E3 ligase Tom1 promotes Yra1 dissociation from Nab2 before mRNP export. These cumulative evidences appoint Nab2 as a new adaptor for Mex67. We propose that in yeast and metazoans Yra1/REF acts as a co-factor stabilizing the adaptor-receptor interaction. The ubiquitin-induced dissociation of Yra1 from mRNAs may ensure nuclear exit of mature mRNAs only.

Poster no. 24.

Mutations of Intronic Retrotransposons in the PTS Gene Lead to Pseudoexon Activation and BH4 deficiency

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Cryptic exons or pseudoexons are typically activated by point mutations that create GT or AG dinucleotides of new 5' or 3' splice sites in introns, often in repetitive elements. Here we describe two cases of tetrahydrobiopterin (BH4) deficiency caused by mutations optimizing the branch point sequence and polypyrimidine tracts and generating repeat-containing pseudoexons in the *PTS* gene. In the first case, we demonstrate a novel pathway of antisense *Alu* exonization, which resulted from an intronic deletion that removed the poly(T)-tail of antisense *Alu*Sq. The deletion brought a favourable branch point sequence within proximity of the pseudoexon 3' splice site and removed an upstream AG dinucleotide required for the 3' splice site repression on normal alleles. New *Alu* exons can thus arise in the absence of poly(T)-tails that facilitated inclusion of most transposed elements in mRNAs by serving as polypyrimidine tracts, illustrating extraordinary flexibility of *Alu* repeats in shaping intron-exon structure. In the other case, a *PTS* pseudoexon was activated by an A>T substitution 9 nt upstream of its 3' splice site in a LINE-2 sequence, providing the first example of a disease causing exonization of the most ancient interspersed repeat. These observations expand the spectrum of mutational mechanisms that introduce repetitive sequences in mature transcripts and highlight the importance of intronic mutations in alternative splicing and phenotypic variability of hereditary disorders.

Poster no. 25.

Dcp1-bodies in mammalian oocytes: a role in translational control?

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The meiotic maturation of mammalian oocytes depends on the translation control of distinct mRNAs for proper completion. Many transcripts especially those related to the cell cycle are quiescent in Prophase I arrested oocytes, and become translationally active upon entry of the oocyte into meiotic maturation. Analysis of the translational profile of one of these transcripts by micro-sucrose gradient revealed the surprising finding that translationally repressed mRNAs can co-localise to the polysomal fractions. Since in somatic cells translationally repressed mRNAs can localise to RNP granules called processing bodies, and we could show that proteins associated with p-bodies also co-fractionate with the polysome, we investigated if p-body like RNP granules are also present during mouse meiotic maturation. Here, we show for the first time the presence of p-body like structures in mammalian germ cells, as revealed by dcp1a localisation and the co-localisation with proteins associated with p-bodies and translational control, namely RAP55 and rck/p54 but not with CPEB1. Two types of foci are observed, large and small, the latter alone being sensitive to cycloheximide and RNase A. We also find that these foci are dynamic during meiotic maturation and that their formation is only marginally dependent on mRNA silencing by small RNAs as described for p-bodies in somatic cells. We are now assessing by FISH and immunoprecipitation if specific translationally repressed mRNAs are present in these p-body like-foci.

Poster no. 26.

Role of microRNA-15a and -16 in cell cycle regulation in non-small cell lung cancer

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MicroRNAs are a recently discovered class of small nucleic acids that regulate gene expression at the post-transcriptional level. This novel class of molecules have essential roles not only in important cellular processes but also in oncogenesis. MiRNA mutations and mis-expression correlate with various human cancers and indicate that miRNAs can function as tumour suppressors or oncogenes.

MiR-15a and miR-16-1 are deleted or downregulated in many tumour types, suggesting that they act as tumour suppressors and are involved in the pathogenesis of tumours. Depending on cell type they induce apoptosis by targeting Bcl-2 [1] or play a role in cell cycle progression [2].

Our research focuses on the biological function of miR-15a and miR-16 in lung cancer cells. Preliminary experiments in lung tissues suggested that the miR-15a/16-1 locus is often deleted in lung cancer what correlates with low miR-15a/16 levels. To investigate if miR-15a/16 is important for non-small cell lung cancer (NSCLC) tumourigenesis or -progression we overexpressed miR-15a/16 in several NSCLC cell lines. This leads to a cell cycle arrest in G₀/G₁ in all NSCLC cell lines tested with the exception of NSCLC-2 cells. In addition we identified cyclin D and cyclin E as new targets of miR-15a/16. Downregulation of these targets may contribute to the miR-15a/16 induced cell cycle arrest. Currently we are investigating the underlying mechanism by which NSCLC-2 cells can escape this cell cycle arrest.

1. **Cimmino et al.** (2005) Proc Natl Acad Sci USA **102**:13944-13949
2. **Linsley et al.** (2007) Mol Cell Biol. **27**:2240-2252