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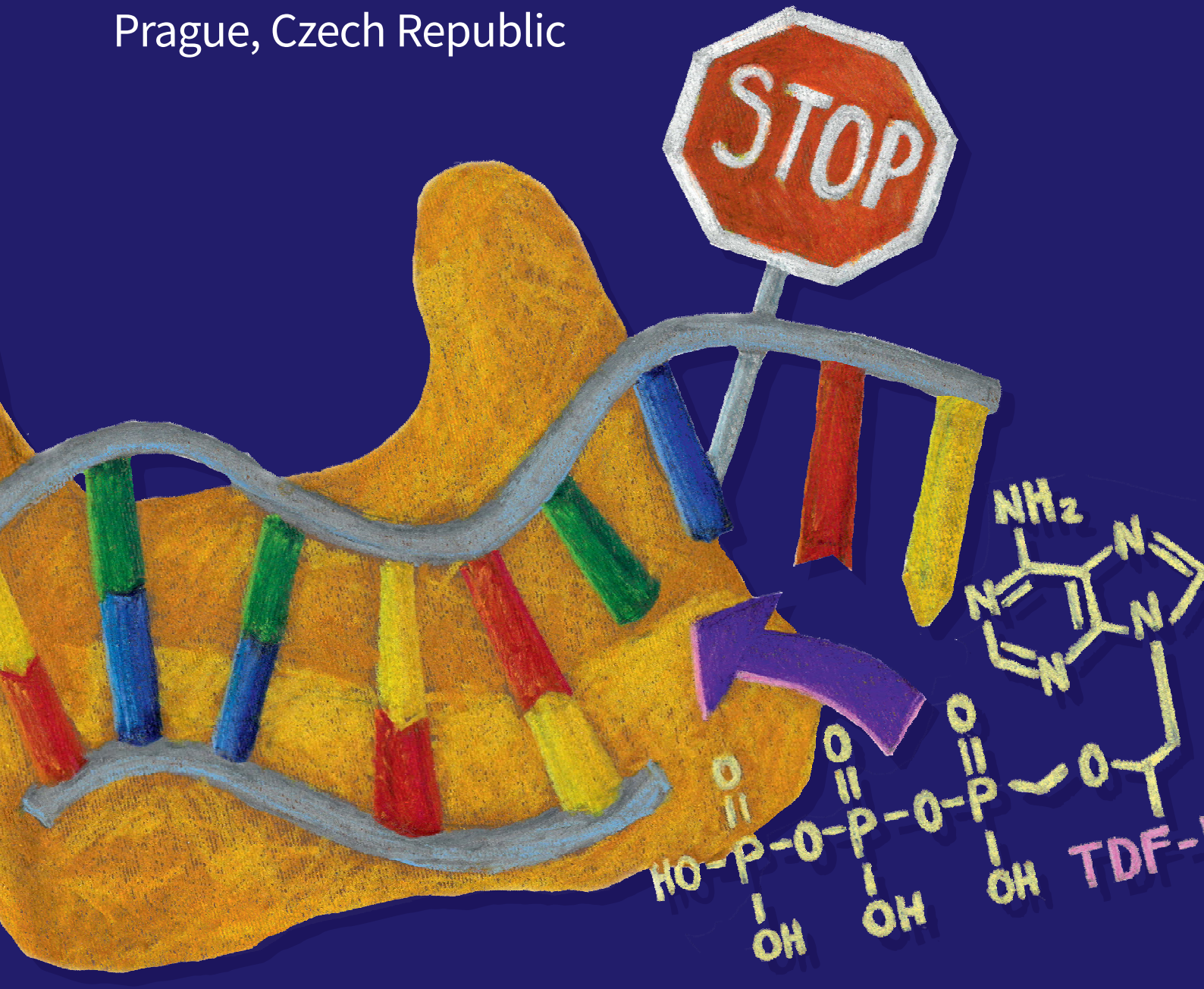

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The abstracts are presented as originally submitted by the authors.

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RNAClub 2019

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IOCB CAS, Flemingovo nám. 542/2, 160 00 Praha 6

RNA Club 2019
September 6, 2019, IOCB CAS, Prague, Czech Republic
Program

8:00-9:00 **Registration**
9:00-9:10 **Opening remarks**

Session 1
Born this way: regulation of gene expression
Chair: Mary O'Connell (CEITEC)

Keynote Lecture 1
9:10-10:10 **Claudia Kutter** (Karolinska Institute, Sweden)
Yin Yang of coding and noncoding transcriptomes

10:10-10:25 **Shubhangini Kataruka** (Svoboda Lab)
miRNA pathway activity in mammalian female germline

10:25-10:40 **Petra Sudzinova** (Krásný lab)
Regulation of rRNA transcription by DNA topology in Bacillus subtilis

10:40-10:55 **Pavel Abaffy** (Šindelka Lab)
Changes in gene expression during dissociation of the tissue into single-cell suspension and preservation of information of original cell state and cell type composition

10:55 -11:25 **Coffee Break**

Session 2
Ch-Ch-Changes: RNA Editing, splicing, and genome modification
Chair: Petr Svoboda (IMG, CAS)

11:25-11:40 **Jiří Sedmík** (O'Connell Lab)
The effects of biallelic variants in ADAR2 on enzymatic activity and their neurological implications

11:40-11:55 **Ales Obrdlik** (Ephrussi Lab)
The Transcriptome-wide Landscape and Modalities of EJC Binding in Adult Drosophila

11:55-12:10 **Michaela Krausova** (Stanek Lab)
Aberrant variants of splicing factor Prpf8 in degenerative transformation of the cerebellum

12:10-12:25 **Martina Hálová** (Folk Lab)
Genetic interactions of PRP45 alleles highlight the dependence of splicing on H2A.Z

12:25-12:35 **Lucie Ulrychová** (BioTech a.s.)
CRISPR/Cas9 & Targeted Genome Editing: New Era in Molecular Biology

12:35-12:45 **Agnieszka Ciesielska** (Bio Rad)
It starts with a droplet and ends in discovery

12:45 -15:00
Lunch and poster session

Session 3
Tattoo'd Lady: DNA and RNA modification
Chair: Michal Hocek (IOCB Prague)

Keynote Lecture 2
15:00-16:00 **Mark Helm** (University of Mainz)
Predict and validate: detection of RNA modifications

16:00-16:15 **Roberto Benoni** (Cahová Lab)
Identification of new class of 5'-RNA caps in E. coli using LC-MS

16:15-16:30 **Pedro Güixens-Gallardo** (Hocek Lab)
Linker-free Bodipy as lifetime fluorescent nucleotide probe: Exploring applications for viral and microRNA detection

16:30-17:00 **Coffee Break**

Session 4
I'll be there for U: RNA-protein friendships
Chair: Štěpánka Vaňáčová (CEITEC)

17:00-17:30 **Zdeněk Paris** (Institute of Parasitology and University of South Bohemia)
Intricate subcellular trafficking of tRNAs in Trypanosoma brucei

17:30-17:45 **Tomas Kouba** (Cusack lab)
Molecular movie of transcribing influenza polymerase

17:45 -18:00 **Ondrej Gahura** (Zikova Lab)
Mitoribosome of Trypanosoma brucei: structural insight into the action and biogenesis of a divergent protein synthesis machine

18:00-18:15 **Kateřina Linhartová** (Štefl Lab)
Recognition of RNA Polymerase II C-terminal domain by RPRD2

18:15-18:30 **Pavla Gajduskova** (Blazek Lab)
CDK11 is required for transcription of replication-dependent histone genes

18:30-18:40
Closing remarks

18:40-22:00
Dinner and party

LECTURES

Keynote lecture 1

Yin Yang of coding and noncoding transcriptomes

Claudia Kutter [1,2]

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The regulation between genetic sequence and transcriptional outcome is essential for understanding species evolution and disease progression. Identifying the divergence and conservation of functional regulatory elements that give rise to a specific transcriptome in a distinct cell at a specific time has been an area of intense investigation. Over the past years it has become apparent that gene expression of protein-coding genes is largely controlled by noncoding RNAs. Our work revealed that noncoding RNAs are key drivers of genome evolution in human and other mammals. In my talk, I will explain that the expression of long noncoding RNAs evolves rapidly and functionally enhances the expression of neighbouring protein-coding genes. In addition, I will present our results on dissecting the functional dependencies of tRNA and mRNA transcriptomes during mammalian evolution, organ development and cancerogenesis. Finally, I will close my talk by showing the regulatory roles of small RNAs.

miRNA pathway activity in mammalian female germline

Shubhangini Kataruka[1], Martin Modrak[2], Veronika Kinterova[3], Daniela Zeitler[4], Radek Malik[1], Jiri Kanka[3], Gunter Meister[4], Petr Svoboda[1]

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4. *Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
5. *Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Libechov, Czech Republic*
6. *RNA Biology, Biochemistry Center Regensburg, University of Regensburg, Regensburg, Germany*

MicroRNAs (miRNAs), genome-encoded 21-23 nt post-transcriptional regulators are functionally important in most somatic tissues but not in mouse oocytes where the miRNA pathway is non-essential and, apparently, non-functional. We hypothesized that the lack of miRNA function stems from unique situation in the oocyte, where the maternal miRNA pool could be too small to significantly regulate the very large maternal transcriptome (~50x larger than a somatic cell transcriptome). The "stoichiometry hypothesis" was tested by quantifying selected miRNAs in mouse oocytes by qPCR and Northern blotting. It showed that while the maternal mRNAs accumulate during oocyte growth, the number of miRNA molecules does not increase and is comparable to miRNA molecule numbers in somatic cells. Surprisingly, quantification of miRNAs revealed the same picture in rat, pig, and cow oocytes. Low miRNA numbers explain miRNA inactivity in porcine and bovine oocytes, as quantified by nanoluciferase miRNA reporter assay. This demonstrates that miRNA inactivity is not mouse oocyte-specific but is a general mammalian phenomenon. Finally, the stoichiometry hypothesis is supported by injection of defined amounts of miRNA mimics, which restores efficient miRNA repression in oocytes of mouse and pig. Consequently, our results also argue against an active repression of miRNA activity in oocytes.

Regulation of rRNA transcription by DNA topology in Bacillus subtilis

Petra Sudzinová[1], Libor Krásný[1]

1. *Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology, the Czech Academy of Sciences, Prague, Czech Republic*

Bacterial cells need to adapt to the changing environment. In a nutrient-rich environment, the cells grow and divide and this requires a large amount of ribosomes. The amount of ribosomes is regulated on the level of transcription initiation of ribosomal RNA (rRNA). In *Bacillus subtilis,* all rRNA promoters are regulated by the concentration of GTP, the initiating NTP (iNTP).

Here, we will demonstrate that rRNA promoters are regulated also by another mechanism than responding to changes in [GTP]. We investigated the role of DNA supercoiling on the activity of rRNA (regulated by [GTP]) as well as control promoters (not-regulated by [GTP]) by *in vitro* and *in vivo* approaches.

In vitro, we observed that changes in supercoiling of rRNA promoters strongly affected the affinity of RNA polymerase (RNAP) for the DNA (initial binding) and iNTP (open complex formation) while in the case of the control P*veg* promoter we observed only altered affinity for the iNTP. *In vivo*, using novobiocin, a compound that affects the state of DNA supercoiling, we showed that rRNA promoter activity changed depending on the DNA topology, whereas P*veg* activity remained the same.

In summary, a new mechanism of regulation of rRNA synthesis is proposed here, depending not only on the concentration of GTP but also on the topological state of DNA in the cell.

This work was supported by the grant No. 19-12956S from the Czech Science Foundation Agency

Changes in gene expression during dissociation of the tissue into single-cell suspension and preservation of information of original cell state and cell type composition

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4. *Laboratory of tumour resistance, Institute of Biotechnology of the Czech Academy of Sciences - BIOCEV, Vestec*
5. *TATAA Biocenter, Göteborg, Sweden*

Analysis of the gene expression at the single-cell level has become very popular during the last few years. The first step of this analysis entails the dissociation of the tissue into a single-cell suspension. Researchers spend alot of time with the optimization of the protocol for library preparation and the proper analysis of the resulting data. However, not much effort has been placed into resolving the problem associated with cells changing expression during the dissociation process. Cells react to the stress caused by dissociation through rapid expression of Stress response genes. These genes include proto-oncogenes such as *Fos* and *Jun* and heat-shock proteins like a *Hsp90aa1*. Unfortunately, these same genes have also been considered markers of tumour progression and their artificial overexpression in the final dataset often lead to wrong data interpretation. Three recent studies [1-3] showed this problem in various experimental setups and proposed possible solutions. However, each of these protocols are using different type of the tissue.

In our experiments, we focused on optimization of dissociation of the mouse mammary tumour. Our goal was not only to preserve the cell state (inhibition of the expression changes during dissociation), but also the cell type composition. We applied RT-qPCR and cell counting to test the effect of different parameters on dissociation effectivity, cell viability and final suspension quality. The data from the "standard" and "optimal" protocols were verified using single-cell RNA-Sequencing to confirm our conclusions. Our experimental design could serve as a muster for other scientists to validate protocols and check functionality on various biological tissues.

1. Adam et al. (2017) Development, 144, 3625-3632.
2. Wu et al. (2017) Neuron, 96, 313-329.
3. Van den Brink et al. (2017) Nat Methods, 14, 935-936.

The effects of biallelic variants in ADAR2 on enzymatic activity and their neurological implications

Jiří Sedmík[1], Mark Fitzgerald[2], Rivka Sukenik-Halevy[3,4], Tiong Tan[5,6,7], Reza Maroofian[8], Mary O'Connell[1]

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2. *Children's Hospital of Philadelphia, Philadelphia, United States of America*
3. *Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel*
4. *Genetics Institute, Meir Medical Center, Kfar Saba, Israel*
5. *Murdoch Children's Research Institute, Melbourne, Australia*
6. *Victorian Clinical Genetics Services, Melbourne, Australia*
7. *Department of Paediatrics, University of Melbourne, Melbourne, Australia*
8. *Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, United Kingdom*

Double-stranded RNA-specific adenosine deaminases (ADARs) are a family of enzymes that catalyse the hydrolytic deamination of adenosine to inosine in dsRNA. The editing and RNA-binding activities of ADARs affect RNA processing, stability, and can even lead to RNA recoding [1]. The enzyme ADAR2 was previously reported to be essential for recoding of transcripts found predominantly in the brain. Impaired ADAR2 editing causes early-onset epilepsy and premature death in mouse models [2].

ADAR2 variants were found in five patients suffering from microcephaly, severe intellectual disability, and seizures. One patient carried a homozygous mutation in one of the double-stranded RNA-binding domains, whereas, in the remaining four patients, homozygous or biallelic mutations located in or around the deaminase domain were identified. To evaluate the effects of these variants on ADAR2 activity, *in vitro* assays with recombinant proteins expressed in HEK293T cells were performed as well as *ex vivo* assays with fibroblasts derived from one of the patients.

Here we will present the results of *in vitro* assays that show reduced editing activity of ADAR2 mutant proteins. We did observe the proper localisation of the mutant proteins. We detected changes in ADAR2 mRNA splicing induced by one of the mutations. Lastly, we observed altered ADAR2 mRNA and protein levels in patient fibroblasts. Together, these results provide evidence that the mutations have a small but significant effect on ADAR2 activity that may lead to the observed phenotype. Proper neuronal model is needed to characterise the effects of these mutations on brain development.

1. Nishikura (2010) *Annu Rev Biochem*, 79, 321-349.
2. Brusa et al. (1995) *Science*, 270, 1677-1680.

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The Transcriptome-wide Landscape and Modalities of EJC Binding in Adult Drosophila

Ales Obrdlik[1,2], Gen Lin[1,3], Nejc Haberman[4], Jernej Ule[4,5], Anne Ephrussi[6]

1. *European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*
2. *CEITEC, Masaryk University, Brno, Czech Republic*
3. *Research Institute of Molecular Pathology (IMP), Vienna, Austria*
4. *Department for Neuromuscular Diseases, UCL Institute of Neurology, London, United Kingdom*
5. *The Francis Crick Institute, London, United Kingdom*
6. *European Molecular Biology Laboratory, Heidelberg, Germany*

Exon junction complex (EJC) assembles after splicing at specific positions upstream of exon-exon junctions in mRNAs of all higher eukaryotes, affecting major regulatory events. In mammalian cell cytoplasm, EJC is essential for efficient RNA surveillance, while in *Drosophila*, EJC is essential for localization of *oskar* mRNA. Here we developed a method for isolation of protein complexes and associated RNA targets (ipaRt) to explore the EJC RNA-binding landscape in a transcriptome-wide manner in adult *Drosophila*. We find the EJC at canonical positions, preferably on mRNAs from genes comprising multiple splice sites and long introns. Moreover, EJC occupancy is highest at junctions adjacent to strong splice sites, CG-rich hexamers, and RNA structures. Highly occupied mRNAs tend to be maternally localized and derive from genes involved in differentiation or development. These modalities, which have not been reported in mammals, specify EJC assembly on a biologically coherent set of transcripts in *Drosophila*.

Aberrant variants of splicing factor Prpf8 in degenerative transformation of the cerebellum

Michaela Krausova[1]

1. *Laboratory of RNA Biology, Institute of Molecular Genetics, Prague, Czech Republic*

Mutations in splicing factors are associated with diverse hereditary syndromes including retinitis pigmentosa (RP) and neurodegeneration. Despite ubiquitous execution of RNA splicing, molecular mechanisms underlying pathognomonic tissue specificity remain elusive. We established two novel murine strains that genocopy RP-linked variants of splicing factor PRPF8. Transcripts carrying the mutations were expressed to levels comparable to wt transcripts and were commonly translated. In parallel, we also generated a *Prpf8* knock-out allele that serves direct comparison with presumptive PRPF8 loss-of-function variants and helps to discriminate between haploinsufficiency and gain-of-function modes. Our pilot phenotypic screening revealed a partial degeneration of retinal layers but notably, both strains developed progressive atrophy of the cerebellum. Starting from two months of age, the cerebellar degeneration proceeds along the mediolateral axis towards vermis and preferentially abolishes granule neurons. In contrast, loss of one *Prpf8* allele does not by itself evoke any pathological phenotype. Moreover, we observed that the postnatal maturation of cerebellum is accompanied by downregulation of several splicing factors including Prpf8, to eventually establish significant gradients of Prpf8 abundance within adult tissue. We thus propose that the cell-autonomous levels of Prpf8 might coincide with the cellular sensitivity to Prpf8 mutations. Collectively, we established novel experimental models that might help to delineate molecular mechanisms associated with aberrant Prpf8 proteins that progressively compromise retinal homeostasis and/or trigger neural death.

Genetic interactions of PRP45 alleles highlight the dependence of splicing on H2A.Z

Martina Hálová[1], Kateřina Abrhámová[1], Martin Převorovský[1], František Půta[1], Petr Folk[1]

1. *Department of Cell Biology, Faculty of Science, Charles University, Praha, Czech Republic*

All stages of gene expression, including transcription and splicing, are affected by histone modifications and nucleosome remodeling. However, this interrelationship is only partly understood. Splicing factor Prp45 from Saccharomyces cerevisiae adopts unusual extended structure with a large portion of predicted intrinsically disordered regions. It forms part of the basic core of the spliceosome through the whole catalytic cycle. It extensively interacts with Prp46 and Prp8 and contacts the U2 snRNP protein Hsh155, RES complex components and NTC factors. SKIP, the human ortholog of Prp45, was shown to be associated with proteins involved in transcription elongation by RNA Pol II, including the transcription factors and their coregulators. C-terminal truncation of Prp45 (prp45(1-169)) is compatible with splicing, but splicing reporters show that both splicing steps are impaired. Cells accumulate high levels of pre-mRNAs, while mRNA levels of most of the intron containing genes are only slightly affected. Nevertheless, the truncation results in impaired cotranscriptional recruitment of U2 snRNP, which is exacerbated in the following spliceosome assembly steps. Remarkably, U1 snRNP recruitment and dissociation are apparently not distorted.

Here, we took advantage of the structural information on spliceosomes and constructed less extensively truncated Prp45 variants which lacked only subsets of the interactions absent in prp45(1-169). Splicing in the new variants was differentially affected, suggesting that Prp45 mediates the association of multiple splicing factors during assembly. We investigated the phenotypes of these prp45 alleles and their dependency on components in cis (the splicing sequence) and in trans (e. g. the factors which affect chromatin). Search for the genetic interactors of prp45(1-169) revealed splicing factors, components of transcription elongation machinery and chromatin modifiers and remodelers. The strongest interactions were found with H2A.Z-encoding htz1, alleles of SWR1, INO80 and SAGA components. prp45 interaction with htz1Δ was stronger than with H2A.Z remodeling complexes tested, and the degree of interaction depended on the extent of Prp45 truncation. Our results support the importance of chromatin environment in pre-mRNA splicing of S. cerevisiae.

CRISPR/Cas9 & Targeted Genome Editing: New Era in Molecular Biology

Lucie Ulrychová [1]

1. *BioTech a.s., Prague*

At this time CRISPR/Cas9 based genome editing is very popular due to it´s efficiency and simplicity. The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material.

The simplicity of the CRISPR system, with only three required components (Cas9 along with the crRNA and trRNA) makes this system amenable to adaptation for genome editing. Based on the CRISPR system was developed and simplified two-component system by combining trRNA and crRNA into a single synthetic single guide RNA (sgRNA). sgRNAprogrammed Cas9 was shown to be as effective as Cas9 programmed with separate trRNA and crRNA in guiding targeted gene alterations. Scientists can synthetize sgRNA with their own fragments, so, it´s possible to use this method to editing various genes in various cells. CRISPR/Cas9 was already used for mice, plants, fishes and many other organisms. It helps to understanding some gene mutations or repairing some mutations in the genome sequences.

For these experiments, company New England Biolabs developed some reagnets, which makes the experiment faster and easier. For example the EnGen sgRNA Synthesis Kit, S. pyogenes provides a simple and quick method for transcribing high yields of sgRNA in a single 30-minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user. EnGen Mutation Detection Kit provides reagents for simple protocol for detecting targeting efficiency in genome editing experiments. And last, but not least NEB offer various types of the Cas nucleases and many other products.

The rapid progress in developing Cas9 into a set of tools for cell and molecular biology research has been remarkable, likely due to the simplicity, high efficiency and versatility of the system. It means that in a few years we´ll be able to heal some diseases like cystic fibrosis or change the character of humans.

It starts with a droplet and ends in discovery

Agnieszka Ciesielska[1]

1. *Field Application Specialist CEE, Bio Rad*

Droplet Digital PCR (ddPCR) is Bio Rad's unique digital PCR technology.

With unrivaled precision, ddPCR provides absolute quantification of target DNA or RNA molecules without the use of standard curves. It addresses the lack of scalable and practical technologies for digital PCR implementation. The new QX200 Droplet Digital PCR System puts this power technology in your hands, ready to unveil new worlds of research at previuosly unattainable levels.

PCR becomes "digital" through sample partitioning and the subsequent statistical analysis of target detection across the partitions. The QX200 System uses advanced microfluidics technology to achieve partitioning on a massive scale, generating 20 000 highly uniform nanoliter-sized droplets per sample. For even higher sensitivity, it is possible to combine wells.
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Keynote lecture 2

Predict and validate: detection of RNA modifications

Mark Helm [1]

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Life Sciences have rediscovered the enhanced structural and functional diversity offered by post-transcriptional RNA modifications. As a previously invisible addition to the conventional transcriptome, modifications form the epitranscriptome. A bottleneck in this very active field is the reliable detection and quantification of modifications, which offers particular challenges in a typical eukaryotic epitranscriptome of 10^7-10^8 residues. Concepts from chemical biology, biophysics, and especially statistics must be tailored to deep sequencing techniques. Successful implementation of the former has led to a number of methods that allow placement of certain modifications within a sequence context, albeit with variegated error rates. For successful application to biological investigations, such methods must be thoroughly validated by orthogonal methods. This presentation features an overview of current techniques in so-called modification mapping, and presents some recent inroads made in the mapping of methylated nucleosides such as m^7G, m^3C, and m^6A.

Identification of new class of 5'-RNA caps in E. coli using LC-MS

Roberto Benoni[1], Oldřich Hudeček[1], Hana Cahová[1]

1. *Chemical biology, IOCB, Prague, Czech Republic*

The role and chemical structure of the 5'-end of prokaryotic RNA is still unclear. The discovery of NAD and Coenzyme A (CoA) [1] as 5' RNA caps changed the perception of the RNA structure. We hypothesized that also other molecules, *e.g.* dinucleoside polyphosphates (Np*n*Ns) can be part of RNA. Np*n*Ns were discovered more than 50 years ago and are present in almost all types of cells [2]. It has been shown that their intracellular concentration can, under stress conditions, increase from the µM to mM range. However, the cellular roles and mechanisms of action of Np*n*Ns are still speculative. They have similar chemical structures as already known RNA caps, such as the nicotinamide adenine dinucleotide (NAD) and 7-methylguanylate cap. Np*n*Ns are excellent substrates for RNA polymerases *in vitro*. Using LC-MS technique we show that both methylated and nonmethylated Np*n*Ns serve as RNA caps in *Escherichia coli.* In total, we identified nine new RNA caps (Ap3A, methylated-Ap3A, Ap3G, methylated-Ap4G, dimethylated-Gp4G, Ap5A, methylated-Ap5A, methylated Ap5G and dimethylated-Ap5G). We analyzed RNA isolated from two growth phases (exponential and late stationary) and we observed significant increase of Np*n*N caps in the late stationary phase. Further, we demonstrated that the *E. coli* decapping enzyme RNA 5' pyrophosphohydrolase (RppH) [3] is able to remove the Np*n*N-caps from the RNA. RppH was, however, not able to cleave the methylated forms of the Np*n*N-caps, suggesting that the methylation adds an additional layer to the RNA stability regulation. Our work introduces an original perspective on the chemical structure of RNA in prokaryotes and the function of RNA caps.

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3. Deana et al. (2008) Nature, 451, 355-358.

Linker-free Bodipy as lifetime fluorescent nucleotide probe: Exploring applications for viral and microRNA detection

Pedro Güixens-Gallardo[1,2], Michal Hocek[1,2]

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2. *Faculty of Science, Charles University, Prague, Czech Republic*

Base-modified fluorescent nucleosides have been very valuable in the study of nucleic acid structure and function. Lifetime fluorescent rotors incorporated in nucleotides showed great potential for displaying microenvironment changes [1-3] We synthesized and studied the photophysical properties of new tiopheneBodipy cytidine (dCtBdp) respect previously reported (dCBdp) [1-3]. Linker free dCtBdp showed considerable advantages when used in oligonucleotides (ONs), recognizing specific RNA and DNA sequences upon hybridization. dCtBdp triphosphate when delivered into the cell [4-5], can easily be distinguished from the *in cellulo* synthesized oligonucleotide bearing the modification using FLIM.

https://ibb.co/tQzVNxh

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This work was supported by the Czech Academy of Sciences (Praemium Academiae to M.H.) and the Czech Science Foundation (17-14791S).

Invited lecture 1

Intricate subcellular trafficking of tRNAs in Trypanosoma brucei

Zdeněk Paris[1,2]

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Proteins and RNAs are routed across the nuclear envelope via the nuclear pore complex and require transport receptors belonging to the karyopherin family (exportins). Only a limited set of export factors, conserved in other organisms, is identifiable in trypanosomes by bioinformatics. Thus our knowledge of tRNA nuclear export in these organisms remains limited. We show here that, like in other eukaryotes, down regulation of the tRNA exporter Xpo-t is not essential in trypanosomes and resulted in neither disruption of mature tRNA export to the cytoplasm nor intron-containing tRNA accumulation in the nucleus; phenotypes commonly observed with analogous yeast mutants. Also similar to yeast, Mex67-Mtr2, the main mRNA transporter in other systems, has a role in tRNA export in *T. brucei* with one major distinction, in *T. brucei* there is a clear separation of functions between Mex67 and Mtr2. The latter still serves a general role as a tRNA and/or mRNA export factor, but down-regulation of its partner, Mex67, leads to the specific accumulation of queuosine (Q)-containing tRNAs in the nucleus. Interestingly, compared to cytosol, tRNAs fully modified with Q, are preferentially imported into the mitochondria. The absence of Q in mitochondria-imported tRNAs also leads to a decrease in *de novo* synthesized mitochondrial proteins implying the role of Q in mitochondrial translation and physiology. Taken together, our data highlights the significance of intracellular trafficking in determining the fate and function of tRNAs via differential modification.

Molecular movie of transcribing influenza polymerase

Tomas Kouba[1], Joanna Wandzik[1], Petra Drncova[1], Stephen Cusack[1]

1. *EMBL Grenoble, Grenoble, France*

Influenza viruses are human pathogens that cause yearly epidemics and occasional pandemics. Influenza virus RNA-dependent RNA polymerase (FluPol) uses unique mechanisms to transcribe its single-stranded genomic vRNA into mRNA and as such is a promising anti-viral drug target [1].

The polymerase is initially bound to a promoter comprising the partially base-paired 3' and 5' extremities of the vRNA. A short, capped primer, 'cap-snatched' from a nascent host polymerase II transcript [2], is directed towards the polymerase active site to initiate RNA synthesis. After processive elongation step, the vRNA transcription is finished by formation of the poly(A) tail by reiterative copying of the 5' proximal oligo-U stretch of the template.

Our extensive biochemical analysis shows that purified recombinant FluPol, when reconstituted with model vRNAs promoter sequences, can perform all steps of cap-dependent transcription *in vitro* [3]. Based on this analysis we are now able to trap distinct RNA synthesis intermediates by varying choice of RNA template and capped primer length and sequence, and by using various NTPs.

Here we present ten structural snapshots, determined by X-ray crystallography and cryo-electron microscopy, of various stages of actively initiating, elongating [4] and terminating FluPol. This revealed unexpected molecular mechanism of the process, e.g. how is the active site cavity unblocked to allow establishment of a ten base-pair template-product RNA duplex, how are template and product strands separated into distinct exit channels, how is the already copied 3' template end retained within the enzyme, and how is the process terminated and recycled.

These extraordinary results allowed us to bring a comprehensive model of the whole process from initiation to termination.

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3. Reich et al. (2017) *Nucleic Acids Res,* **45**, 3353-3368.
4. Kouba et al. (2019) Nat Struct Mol Biol, **26**, 460–470.

Mitoribosome of Trypanosoma brucei: structural insight into the action and biogenesis of a divergent protein synthesis machine

Ondrej Gahura[1], Shintaro Aibara[2], Victor Tobiasson[3], Martina Slapnickova[1], Alena Zikova[1], Alexey Amunts[4]

1. *Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic*
2. *SciLifeLab, Stockholm University, Stockholm, Sweden*
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Mitochondrial ribosomes (mitoribosomes) diverged considerably not only from their prokaryotic and cytoplasmic counterparts, but also between eukaryotic lineages, reflecting differential requirements of the vestigial genomes of the organelle. Mitoribosomes of *Trypanosoma brucei*, a parasitic protist belonging to the early branching lineage of Excavata, are characterized by an exceptionally reduced RNA content and expanded repertoire of proteins [1].

We purified the trypanosoma mitoribosome from native source and determined its structure by single particle cryoEM. The resulting atomic model corresponds to the structure of *T. brucei* mitoribosomes isolated by tandem affinity purification published recently [2]. In addition to the 3D-models of the entire mitoribosome and its large and small subunit (LSU and SSU), our large cryoEM dataset yielded sub-3.5 Å resolution maps of an unexpected variety of mitoribosome-related complexes, among others the LSU and SSU assembly intermediates and the preinitiation complex. Atomic models of these complexes provide wealth of structural information essential for understanding of the pathways of trypanosome mitoribosome biogenesis and recycling. In total, we identified at least 50 lineage-specific or conserved assembly factors, enzymes for RNA modification and remodelling, and a number of unannotated genes' products, including mitochondrial translation initiation factors.

Our data reveal unique features of the architecture and assembly of the trypanosome mitoribosomes, which co-evolved with the atypical mitochondrial gene expression pathway in this organism. At the same time, the comparison of ribosomes and related complexes from *T. brucei* mitochondria with structures of bacterial, cytoplasmic and other mitochondrial ribosomes enable determination of invariable traits of protein synthesis machineries.

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Recognition of RNA Polymerase II C-terminal domain by RPRD2

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The largest subunit of human RNA Polymerase II contains highly flexible C-terminal domain (CTD) that is composed of 52 heptapeptide repeats (21 repeats with consensus sequence YSPTSP and 31 nonconsensual repeats). Several CTDs canonical and non-canonical residues can be subjects of post-translational modifications. Tyrosine, threonine and serine residues undergo dynamic phosphorylation/dephosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These phosphorylation patterns are recognized by various transcription and processing factors during the transcription cycle. Therefore, CTD plays an important role in the regulation of transcription and coupling of transcription to post-transcriptional processes such as mRNA processing.

In this study, we show that human transcription factor, RPRD2, recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting domain (CID) in a similar way to its yeast homologue, Rtt103. The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID and pSer2,7 CTD, the solution structure was obtained using NMR spectroscopy. pSer 2 and pTh4 phosphomarks occur mainly during the late elongation and termination. RPRD2s preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination.

CDK11 is required for transcription of replication-dependent histone genes

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Expression of canonical, replication-dependent histones (RDH) is highly upregulated during S-phase when they package the newly synthesized DNA. We used iCLIP, which identifies protein-RNA interactions at nucleotide resolution, to study the function of the cyclin-dependent kinase 11 (CDK11), an essential gene in several cancers. CDK11 directly binds RNA via its N-terminal region, which lacks any canonical RNA binding domain. It predominantly binds the RDH mRNAs, and is required for their efficient expression. ChIP-seq revealed strong enrichment of CDK11 on RDH genes, with strongest binding in S phase, which depended on RNA and active transcription. CDK11 phosphorylates serine 2 (Ser2) of the C-terminal domain (CTD) of RNA polymerase II (RNAPII) and its depletion reduced RNAPII association with RDH genes. Altogether, CDK11 acts as a Ser2 CTD kinase specifically at RDH genes to control their optimal transcription.

POSTERS

The African Swine Fever Virus RNAP Transcription System

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The African Swine Fever Virus (ASFV) is a nucleocytoplasmic large dsDNA virus (NCLDV) that causes lethal and incurable haemorrhagic fever in pigs. ASFV carries out RNA transcription and modification independently of host cell machinery, as the ASFV genome encodes an 8-subunit RNA polymerase (RNAP), a poly-A polymerase, and mRNA capping enzyme. Strikingly, the ASFV transcription system, encompassing ASFV-RNAP and general transcription factors, is reminiscent of the RNAP-II-like system. ASFV utilises a combination of divergent homologs of the basal factors TATA-binding protein (TBP) and transcription factor II B (TFIIB) for transcription initiation, along with Vaccinia virus-like viral transcription factors/regulators. However, the underlying molecular mechanisms of ASFV-RNAP and its factors are unknown, as is the promoter architecture and DNA motifs including TATA-boxes and B-recognition elements that could serve as binding sites for TBP and TFIIB, respectively.

We have applied a combination of deep sequencing techniques including CAGE and RNA-seq to determine (i) the global transcription start site (TSS) map and (ii) the transcriptome of the ASFV strain BA71. We could assign the primary TSSs of 151 (out of the 153) ASFV genes with high confidence. This enabled the correct annotation of ORFs with alternative start codons and the prediction of novel ORFs and antisense RNA. Multiple sequence alignments and MEME motif searches of DNA sequences immediately upstream and downstream of the TSSs revealed a distinct 11-nucleotide motif (ATAAAATT[T/G]AA) likely corresponding a promoter element. This consensus sequence and its location relative to the TSS is similar to the Pox virus early promoter core motif and the promoter signature in the yeast Killer plasmids, and not similar to canonical eukaryotic BRE or TATA boxes. RNA-seq transcriptome mapping highlighted the abundance of poly-U stretches at the 3’ ends of mRNAs, suggestive of a mechanism of transcription termination reminiscent of factor-independent, intrinsic, termination. We furthermore characterised the transcriptome of ASFV-infected Vero cells at 5 hours and 16 hours post-infection, which showed that 103 genes were differentially expressed in the early and late stages of infection. Our data also provided insight into the most highly-expressed genes including uncharacterised viral proteins which could act as potential targets in future vaccine developments.

Analysis of the protein network of mammalian m6A/m6Am methylases and demethylases

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Eukaryotic RNAs can carry more than 100 different types of chemical modifications. The importance of 5' capping or 3' polyadenylation of mRNAs has been revealed several years ago, but the roles of other modifications are just starting to be understood. N6-methyladenosine (m6A) and the di-methylated form found next to the 5'-cap N6,2'-O-methyladenosine (m6Am) are two abundant modifications found in mRNAs widely studied during the last years. Writer, eraser and reader proteins regulate the deposition, removal and recognition of these chemical marks modulating almost every aspect of RNA metabolism. In mammals, m6A is deposited either by the methyltransferase complex METTL3/METTL14 or by METTL16 and two proteins catalyze its removal, ALKBH5 -specific for m6A- and FTO -targeting both modifications. However, whether these methylases and demethylases work in independent pathways or some of them cooperate to perform specific functions remains elusive. We aim to address this question by analyzing and comparing the transient protein interactome of METTL3, METTL16, ALKBH5 and FTO obtained by BioID (proximity-dependent biotin identification) [1] in Hek293 cells. We prepared stable cell lines expressing BirA*-tagged versions of the proteins and performed *in vivo* labelling and protein pulldown followed by total protein analysis by mass spectrometry. Using this approach, we have generated a complete dataset of proteins connected with the main players of the m6A pathway in mammalian cells.

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Retinitis pigmentosa linked mutation of Prpf8

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Prpf8 is a highly conserved pre-mRNA splicing factor and a crucial component of the U5 snRNP. Mutations in Prpf8 were found in patients with retinitis pigmentosa (RP), a human disease caused by a loss of photoreceptors in the retina and degenerative changes in the retinal pigment epithelium (RPE). Previously, we showed that majority of RP-linked mutations impair incorporation of Prpf8 into splicing complexes and inhibit splicing. However, this does not apply to the protein carrying the Y2334N mutation, which was properly incorporated into splicing complexes and splicing defects were gene specific (Malinova et al., *JCB*, 2017).

Here, we utilized CRISPR/Cas9 system to tag endogenous Prpf8 with GFP and to introduce Y2334N mutation in the near-diploid RPE-1 cell line. We prepared homozygotic and heterozygotic cell lines expressing GFP tagged Prpf8 or the Y2334N mutant. The Y2334 mutant is localized to splicing speckles similarly to wild type. We further confirmed by immunoprecipitation that the Y2334N mutant is properly incorporated into snRNPs. In a mouse model, Prpf3 mutation reduced phagocytic abilities, which might impair photoreceptor function in retina. We therefore probe whether the mutation in Prpf8 also impairs phagocytosis of human RPE cells.

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Age related differences in the translational landscape of mammalian oocytes

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Oocyte aneuploidy is the result of abnormal chromosome segregation during meiosis giving rise to a ready to be fertilized oocyte which, however, possesses an aberrant number of chromosomes. These anomalies are inherited by the future embryo drastically reducing its developmental potential. Furthermore, aneuploidy is not an infrequent event in mammalian oocytes but rather a common feature, the frequency of which increases in correlation with female age. Importantly, maturing oocytes are transcriptionally silent and rely on the utilization of a pool of mRNAs synthesized and stored during the growth period. We have applied a polysome fractionation method to isolate RNA population involved in the active translation during maturation from as little as 200 oocytes from both young and aged mouse females. After sequencing through Illumina NextGen, we have identified several genes with potential different expression levels between oocytes from young and aged females and therefore might intervene in age-related aneuploidy. Moreover, gene ontology enrichment analysis of polysome-bound RNAs reveal biological function categories related with translation initiation and regulation with higher incidence on oocytes from aged females. This results shed some light upon the reasons behind the genomic instability or loss of quality in oocytes from women of advanced age.

Searching for DIS3L2 partners in crime

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RNA surveillance and degradation pathways belong to the most important cellular mechanisms to ensure proper RNA maturation and function. The 5’ to 3’ exoribonuclease DIS3L2 is one of the enzymes involved in cytoplasmic quality control of broad spectrum of RNA molecules, most of which are miss-processed and share stable secondary structures. RNA molecules targeted by TDS (TUT-DIS3L2 surveillance) are tagged by a 3’ oligo(U) tail by on of the Terminal Uridine Transferases. Oligouridylation of RNA seems to be the only specific mark by which DIS3L2 recognizes the RNA molecules destined for trimming or decay.

It is unclear whether the main targetting specificity of DIS3L2 is determined by the uridylation status of RNAs or whether additional factors regulate RNA binding and trimming efficiency. So far, only RNA-dependent co-immunoprecipitation with the 5' to 3' exoribonuclease XRN1 [1] and TUT1-Ago2 complex [2] was shown. However, the attempts of several labs did not reveal any additional stable interactors to date.

To tackle the stable and transient DIS3L2 interactome, we use the combination of proximity-dependent biotin identification (BioID) coupled to quantitative MS/MS analysis [3]. The preliminary data from this experiment will be presented and discussed.

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Manipulation of transcription by bioorthogonal chemistry

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Recent advances in imaging of transcription *in vivo* are calling for a development of techniques to precisely manipulate activity of particular genes. One means to perform such manipulations is the introduction of artificial epigenetic modifications to DNA bases with inhibitory effects on transcription that can be subsequently removed by bioorthogonal reactions, i.e. chemical reactions which can take place inside living systems. In our group, an enhancement of transcription by 5-hydroxymethyluracil (hm-U) and 5-hydroxymethylcitosine (hm-C) was observed in bacterial systems while the DNA templates containing nitrobenzyl modified uracil or cytosine (NB-U, NB-C) were blocked for transcription. In collaboration with Michal Hocek´s group from IOCB AS CR, we developed an *in vitro* approach for switching transcription ON by uncaging the NB modified DNA using irradiation by 400 nm light. Such reactions removed NB and the resulting hm-U or hm-C modified DNA could be readily transcribed by the bacterial RNA polymerase. Here, we present results of the next step where we aimed to perform this photoswitching of transcription within living mammalian cells. We created a model promoter-*gfp* fusion construct, and modified the DNA with hm-U, hm-C, NB-U or NB-C. Then, we observed that the hm-U and hm-C modified DNA molecules were transcribed and translated to GFP although at a lower level than the unmodified DNA. In contrast, the presence of NB groups had an inhibitory effect on GFP expression in mammalian cells. Future experiments will address the uncaging of NB-modified templates. In summary, these results promise manipulations of gene expression in living cells via bioorthogonal chemistry in the future.

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Nuclear tRNA export in Trypanosoma brucei

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Transfer RNAs (tRNAs) are essential components of the cells performing an important function in protein synthesis. Protein and RNA transport across the nuclear envelope occurs through the nuclear pore complex (NPC) and requires proteins of the karyopherin family (exportins). Exportin-t (Xpo-t) in vertebrates and Los1 and Msn5 in yeast specifically export tRNAs from the nucleus; however, deletion mutants of these proteins are viable suggesting functional redundancy. *T. brucei* is a single-cell parasite causing sleeping sickness in humans that, in contrast with other eukaryotes, lack most of the transcriptional control; the bulk of gene expression regulation occurring post-transcriptionally. Nuclear tRNA export might provide an additional level of regulation of gene expression during the complex life cycle of these parasites. However, only a limited set of the export factors, conserved in other organisms, can be easily identified in the *T. brucei* genome; thus our knowledge of nuclear tRNA export remains limited. Our data shows that typical tRNA exporters for yeast or vertebrate are also unessential for growth of *T. brucei.* Moreover, the RNAi silencing of TbXpo-t did not result neither in disruption of the tRNA translocation, nor in the intron-tRNA accumulation in the nucleus, both phenotypes described for yeast mutants. However, our recent data show that the heterodimeric export receptor Mex67-Mtr2, the main transporter for mRNA, has a role in tRNA export. Silencing of Mex67-Mtr2 led to accumulation of mature spliced tRNATyr providing another evidence for the existence of the tRNA retrograde pathway in *T. brucei*. In addition, the level of queuosine modified tRNA is influenced by the efficiency of the tRNA nuclear export by Mex67-Mtr2. This can be explained by the prolonged availability of the substrate (tRNA) to the enzyme TbTGT1/2 in the nucleus. Taken together, these data provide a possible link between the tRNA trafficking and regulation of translation.

In vitro analysis of RNA binding properties of the human DIS3L2 exoribonuclease

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DIS3L2 is eukaryotic 3‘ – 5‘ exoribonuclease. It is a homolog of the main catalytical exosome subunit, yeast Rrp44 and mammalian DIS3 and DIS3L. DIS3L2 specifically binds to a wide variety of oligouridylated aberrant RNAs and facilitates their trimming and decay [1-4]. Most of the *in vivo* RNA targets are highly structured noncoding RNAs.

The crystal structure of incomplete mouse DIS3L2 in complex with oligoU RNA revealed its specificity for UMP residues [5]. However, detailed mechanism of RNA recognition and of the RNA unwinding activity of DIS3L2 remains unclear. Structural analysis is complicated by the intrinsically disordered parts of DIS3L2, which are mainly at both C- and N- termini and make up to 30 percent of the protein [5].

In this work, we focus on the role of the disordered parts of the protein in RNA binding and DIS3L2 catalysis. Using *in silico* analysis such as the tertiary structure prediction, surface electrostatics prediction, amino acid residues conservation and point mutations found in patients we predicted several sites suitable for *in vitro* mutagenesis and further analysis.

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Dinucleoside polyphosphates as novel type of 5'-RNA caps – biosynthesis and biodegradation

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In eukaryotes, the 5'-end of mRNA is protected by 7-methylguanylate cap (m7G). Beside its protective role against RNA degradation, the m7G cap is recognised by numerous biomolecules, e.g. by the translation initiation factor. Recently, it was found that also bacteria possess capped RNA. The nicotinamide adenine dinucleotide (NAD) is attached to 5'-end of some regulatory small RNAs (sRNA) and sRNA-like 5'-terminal fragments of certain mRNAs [1]. As the dinucleoside polyphosphates (Np*n*N) have similar structure to already known 5'-RNA caps, we assumed that they also might be incorporated into RNA. The Np*n*Ns are pleiotropic molecules present in almost all types of cells and they were discovered more than 50 years ago [2]. Their concentration in the cells increases under stress conditions, therefore they are called alarmones. However, their cellular roles and mechanisms of action are still unclear. We are the first to show that Np*n*Ns (Ap3-6A, Ap4-5G, Gp4G) are excellent substrates for T7 and *E. coli* RNA polymerases (RNAP) and efficiently initiate *in vitro* transcription [3]. We also studied how good substrates are Np*n*Ns for RNAP in the presence of a constant ATP and GTP concentration. Further, we demonstrated that the *E. coli* decapping enzyme RNA 5' pyrophosphohydrolase (RppH) [4-5], belonging to the NudiX enzyme family, is able to remove the Np*n*Ns-cap from the RNA. To understand the substrate specificity of RppH, we performed kinetic study of all Np*n*N-capped RNAs. In addition to that, we confirmed by LC-MS analysis that both methylated and non-methylated NpnNs serve as RNA caps in *Escherichia coli*. All the *in vitro* experiments and kinetic studies will be discussed in this poster.

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Synthesis and Degradation of Ms1 in Mycobacterium smegmatis

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Microorganisms have to adapt to changing environments and this is mediated by changes in gene expression. Small non-coding RNAs (sRNAs) are involved in regulation of this process. An example of such a sRNA is Ms1, which was found in *M. smegmatis* in stationary phase. Its homologs are present among mycobacteria including *M. tuberculosis* or *M. bovis*. Ms1 forms a complex with the RNA polymerase core and it enhances cell survival under various types of stress. In stationary phase, Ms1 is highly stable; however, it is rapidly degraded after dilution to nutrient-rich medium. The accumulation of Ms1 in stationary phase depends both on its synthesis and degradation rates, but the specific mechanisms remain unknown. We identified and characterized the Ms1 promoter, the dynamics of Ms1 expression, and the presence of a transcription factor required for its efficient expression. Further, we identified an RNase, polynucleotide phosphorylase (PNPase), as an interacting partner of Ms1. Based on RNAseq data, PNPase is expressed ~10x more in exponential than in stationary phase, which inversely correlates with the accumulation dynamics of Ms1. Finally, we performed *in vitro* experiments that revealed the effect of recombinant PNPase on Ms1 degradation. In summary, we provide a comprehensive characterization of the control of the intracellular Ms1 level, paving the way to clarifying the mechanism of Ms1 synthesis and degradation in mycobacteria.

This work is supported by grant No. 17-03419S from the Czech Science Foundation.

SART3 binding to post-splicing snRNPs suggests a molecular mechanism for spliceosome recycling

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Human spliceosome is a complex and dynamic machine composed of hundreds proteins and five small nuclear RNAs (snRNAs). Core spliceosomal proteins are organized around these snRNAs to form small nuclear ribonucleoproteins (snRNPs) that are named according to their respective snRNA: U1, U2, U4, U5 and U6. During activation of the spliceosome, snRNPs are remodeled to catalyze the splicing reaction. Post-spliceosomal snRNPs then need to be recycled to their original shape before a next round of splicing. Currently, there are very few data available about snRNP recycling and factors involved in this process. Protein SART3 has been proposed to function as a U4/U6 snRNP recycling factor, however a molecular mechanism of its function is not known. Here, we have analyzed SART3 interactions with spliceosomal snRNPs and discovered unexpected binding between SART3 and core U2 snRNP. Using MS2 system, we immunoprecipitated specifically U2 particles stalled prior to spliceosome assembly. These pre-spliceosomal U2 snRNPs do not associate with SART3 suggesting that SART3-U2 snRNP interaction does not occur during U2 snRNP biogenesis but is restricted to the spliceosome or recycled snRNPs. To characterize SART3-bound complexes, we applied two-step immunoprecipitation to specifically enrich particles containing both SART3 and U2 snRNPs. We show that in addition to U2 snRNP, immunopurified SART3-U2 complexes also contain U5 and U6 snRNAs. Together, our data imply that SART3 interacts with post-catalytic spliceosomes composed of U2, U5 and U6 snRNPs. We speculate that SART3 binds to a post-splicing complex to facilitate the release of U6 snRNA and chaperones U6 during its recycling.

A GTP-dependent switch that controls G-quadruplex multimer formation

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G-quadruplexes are four-stranded nucleic acid structures typically made up of stacked GGGG tetrads connected by short loops. Although most studies investigating potential biological roles of G-quadruplexes have focused on monomeric structures, recent work suggests that multimeric G-quadruplexes could also be important [1]. We recently identified mutations in the central tetrad of a monomeric G-quadruplex that induce formation of higher-order structures [2-4]. Here we show that both DNA-DNA and DNA-RNA G-quadruplexes containing a guanosine to adenosine mutation at a specific position in this tetrad behave like molecular switches in which the equilibrium between monomeric and multimeric G-quadruplex is controlled by GTP concentration [5]. Analysis of the nucleotide specificity of inhibition and characterization of the mechanism of binding by NMR suggest that GTP stabilizes the monomeric form of the G-quadruplex by becoming incorporated into one of the tetrads. Hundreds of sequences with the potential to form such GTP-dependent switches are present in the human genome, including some that are evolutionarily conserved. Our experiments provide new insights into the small molecule-mediated control of G-quadruplex multimerization, and raise the possibility that a GTP-dependent switch controls G-quadruplex multimer formation in cells.

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Recognition of RNA Polymerase II C-terminal domain by RPRD2

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The largest subunit of human RNA Polymerase II contains highly flexible C-terminal domain (CTD) that is composed of 52 heptapeptide repeats (21 repeats with consensus sequence YSPTSP and 31 nonconsensual repeats). Several CTDs canonical and non-canonical residues can be subjects of post-translational modifications. Tyrosine, threonine and serine residues undergo dynamic phosphorylation/dephosphorylation resulting in specific phosphorylation patterns throughout different stages of transcription cycle. These phosphorylation patterns are recognized by various transcription and processing factors during the transcription cycle. Therefore, CTD plays an important role in the regulation of transcription and coupling of transcription to post-transcriptional processes such as mRNA processing.

In this study, we show that human transcription factor, RPRD2, recognizes specifically pSer2 or pThr4 phosphorylated forms of CTD via its CTD-interacting domain (CID) in a similar way to its yeast homologue, Rtt103. The interaction of RPRD2 CID with pSer2 phosphorylated CTD is further enhanced by additional phosphorylation on pSer7. To provide mechanistic details of the interaction between RPRD2 CID and pSer2,7 CTD, the solution structure was obtained using NMR spectroscopy. pSer 2 and pTh4 phosphomarks occur mainly during the late elongation and termination. RPRD2s preference for these two phosphomarks suggests possible involvement of RPRD2 in transcription termination.

The role of RNA-specific terminal transferase PAPD7

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RNA-specific ribonucleotidyl transferases are polymerases that modify RNA molecules by adding ribonucleotides to their 3´ ends. PAPD7, an enzyme with putative 3´ poly(A) transferase activity, is involved in a post-transcriptional quality control mechanism. Papd7 is ubiquitously expressed with an exceptionally high expression during the oocyte-to-zygote transition, which makes it a great candidate for factor regulating gene expression during this process. To investigate the function of PAPD7, we used a conditional knock-out ESC line from gene knock-out consortium EUCOMM and generated whole mouse knock-out lacking a predicted critical exon from the gene structure. Knock-out mice are viable; females show minimal fertility phenotype; however, we observe impairment of spermatogenesis and male sterility. Notably, we have found that the mutant Papd7 allele lacking the critical exon can still produce truncated PAPD7 containing just one of the two annotated functional domains. The truncated PAPD7 appears to reduce the Papd7 mutant phenotype and provides a model for uncoupling biological roles of the N- and C-terminal parts of the protein.

CDK12 controls G1/S progression by regulating RNAPII processivity at core DNA replication genes

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CDK12 is a kinase associated with elongating RNA polymerase II (RNAPII) and is frequently mutated in cancer. CDK12 depletion reduces the expression of homologous recombination (HR) DNA repair genes, but comprehensive insight into its target genes and cellular processes is lacking. We use a chemical genetic approach to inhibit analog-sensitive CDK12, and find that CDK12 kinase activity is required for transcription of core DNA replication genes and thus for G1/S progression. RNA-seq and ChIP-seq reveal that CDK12 inhibition triggers an RNAPII processivity defect characterized by a loss of mapped reads from 3'ends of predominantly long, poly(A)-signal-rich genes. CDK12 inhibition does not globally reduce levels of RNAPII-Ser2 phosphorylation. However, individual CDK12-dependent genes show a shift of P-Ser2 peaks into the gene body approximately to the positions where RNAPII occupancy and transcription were lost.
Thus, CDK12 catalytic activity represents a novel link between regulation of transcription and cell cycle progression. We propose that DNA replication and HR DNA repair defects as a consequence of CDK12 inactivation underlie the genome instability phenotype observed in many cancers.

Enzymatic synthesis of base-modified RNAs

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Base modified RNAs are indispensable tool for studying RNA structure and dynamics [1]. Routinely used enzyme for in vitro RNA transcriptions is T7 RNA polymerase, since it does not require any special conditions nor the presence of transcription factors for its function [2]. However, despite many work done on incorporation of specific modified triphosphates by RNA polymerase, there was no systematic study and comparison of substrate activity of NTPs bearing modifications at pyrimidine or 7-deazapurine bases. To address this issue, we prepared the library of eighteen 5-substituted pyrimidine or 7-substituted 7-deazapurine nucleoside triphosphates bearing modifications of different size: methyl, ethynyl, phenyl, benzofuryl and dibenzofuryl group, using aqueous cross couplings directly on triphosphates or on the nucleosides followed by phosphorylation. We have then systematically studied the influence of the modification on the efficiency of in vitro transcription and found that modified ATP, UTP and CTP analogues bearing smaller modifications were good substrates for the RNA synthesis even in difficult sequences incorporating multiple modified nucleotides. Bulky dibenzofuryl derivatives of 7-deazapurine triphosphates were not substrates for the RNA polymerase. In case of modified GTP analogues, a modified procedure using a GMP as initiator needed to be used to get efficient RNA synthesis. The T7 RNA polymerase synthesis of modified RNA can be very efficiently used for synthesis of modified RNA but the method has constraints in sequence of the first three nucleotides of the transcript, which must contain a non-modified G in the +1 position [3].

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A missense DDX38 mutation linked with retinitis pigmentosa

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During splicing, snRNPs and pre-mRNA undergo a series of association-dissociation steps and eight RNA helicases play essential roles in controlling these conformational rearrangements. A large number of human diseases are consequence of splicing errors. Surprisingly, most mutations in ubiquitously expressed spliceosome components exhibit a tissue specific phenotype. For example, mutations in several snRNP-specific proteins cause retinitis pigmentosa (RP), a major cause of blindness. A missense mutation in the RNA splicing helicase DDX38, which results in the Gly332Asp substitution was associated with early-onset autosomal recessive RP. Our aim is to determine how RP mutation affect DDX38 function. We provide evidence that the DDX38 helicase interacts with several components of the active spliceosome and that the RP mutation does not inhibit these interactions. We further show that the knockdown of DDX38 as well as the expression of RP-related mutant protein affect splicing efficiency of several ubiquitously expressed and retina specific genes. Moreover, we showed that the knockdown of DDX38 and expression of the mutant variant enhance usage of cryptic splice sites. We hypothesize that DDX38 has role in RNA splicing quality control and that RP-related mutation affects this function.

Analysis of cell-free circulating ribosomal RNA in human plasma

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RNA is not confined to individual cells only, but as was discovered in recent years, it is also part of different types of extracellular vesicles secreted by cells including ectosomes, exosomes or oncosomes or attached to secreted proteins.

Therefore, extracellular RNA's (exRNA) potential to define states of cells in various healthy or diseased tissues is extensively explored. In the literature, it was claimed that exRNA contains only minute amounts of ribosomal RNA (rRNA). However, when we sequenced exRNA isolated from cell-free human plasma, we found out that rRNA comprise almost 80% of exRNA. Thus, we decided to investigate the expression of rRNA present in human cell-free plasma.

Total exRNA isolated from human cell-free plasma was sequenced and then aligned to Human ribosomal DNA complete repeating unit sequence (GeneBank U13369.1). Individual features present in this sequence - 5’ and 3’ external transcribed spacers (ETS), internal transcribed spacer (ITS) and 5.8S, 18S and 28S rRNAs - were quantified and per base coverage calculated.

We found, that 18S and 28S rRNAs were present at high comparable levels, while 5.8S rRNA expression was almost 10-fold lower. ETS and ITS were also detectable, although at minimal levels. Interestingly we found out that the coverage of each of these rRNAs was uneven without clear 3’ or 5’ bias and we were unable to relate this variation to secondary structure.

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NAD capping in RNA in E. coli and M. smegmatis

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Nicotinamide adenine dinucleotide (NAD) capping is a newly discovered 5'-end RNA modification identified in different types of transcripts, including regulatory small RNAs, in bacteria, yeast, plant, and human cells. This RNA-modification is best characterized in *Escherichia coli*, and has never been studied in the clinically relevant mycobacteria. *Mycobacterium smegmatis* is often used due to its similarity to the lethal human pathogen *M. tuberculosis*. Given the key role of the 5'-end for stability and functionality of RNA, it is of particular interest to define the targets and effects of NAD-capping in *M. smegmatis*. Towards this goal, we have identified the NAD-capped transcripts in *M. smegmatis*, as well as discovered new promoter determinants for NAD-capping and performed experiments shedding light on NAD-cap's biological role in *E. coli*.

Domain functions of Delta subunit of Bacillus subtilis RNA polymerase

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Bacterial RNA polymerase (RNAP) is the key enzyme responsible for transcription of DNA into RNA. RNAP is an extensively studied multisubunit complex. The RNAP core consists of α2ββ'ω subunits. Unlike gram-negative bacteria, the RNAP core from Bacillus subtilis and other gram-positive Firmicutes contain an additional subunit, delta.

The delta subunit is composed of two domains: the structurally well-organized N-terminal and the unstructured highly acidic C-terminal domain, both domains are connected by highly conserved positively charged Lysine tract. Recent studies mainly focused on functioning of the whole delta subunit. The mechanistic functioning of individual domains is still not fully defined. Here, we will present results of experiments that address functions of the two domains of delta and a role of Lysine tract studied by in vitro transcriptions, in vivo competitive cell growth assays and structural analysis of NMR and SAXS data obtained for delta and its mutant. Our results show the profound effect of presence of the C-terminal domain on transcription regulation and on cell survival when facing a competing strain. Implications of these results for the functioning of the delta protein and the transcription machinery will be discussed.

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MoaB2, new interaction partner of σA in Mycobacterium smegmatis

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Bacterial RNA polymerase (RNAP) contains the catalytic core ($\alpha_2\beta\beta'\omega$) associated with one of several sigma (σ) factors, which determine promoter recognition. Here, we identify MoaB2 as a novel binding partner of the mycobacterial primary sigma factor, σA. Furthermore, we present an initial characterization of this transcription factor: (i) MoaB2 is not essential, (ii) MoaB2 affects mycobacterial transcription *in vitro,* and (iii) MoaB2 and σA mutually affect amounts of their mRNA levels *in vivo* (decreased moaB2 mRNA level results in increased σA mRNA level and *vice versa*). In summary, a new transcription factor has been discovered, and this knowledge may be utilized in future designs of novel antimycobacterial drugs.

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LC/MS analysis and deep sequencing reveal the accurate RNA composition in the HIV-1 virion

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Viruses are a major force that shapes the evolution of both pro- and eukaryotic organisms. The mechanism of action of various viruses has been the primary focus of many studies. Yet, the data on RNA modifications in any type of virus are scarce. Methods for the sensitive analysis of RNA modifications have been developed only recently and they have not been applied to viruses. In particular, the RNA composition of HIV-1 virions has never been determined with sufficiently exact methods. This study reveals that the RNA of HIV-1 virions contains surprisingly high amount of the 1-methyladenosine (m1A). We used a liquid chromatography-mass spectrometry analysis (LC/MS) of virion RNA combined with m1A profiling and deep sequencing to recalculate the RNA composition of HIV-1 virion. Moreover, we present a promising new tool for studying the RNA compositions of virions in general.

Comprehensive splice-site analysis of marine Diplonemids using comparative genomics approach

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RNA splicing plays a critical role in regulating gene expression and transcriptome diversity in a variety of eukaryotes. Recent global surveys of marine biodiversity revealed that planktonic diplonemids are amongst the most abundant and diverse marine organisms. These so-far overlooked protists related to important kinetoplastid parasites (Trypanosoma, Leishmania) as well as ubiquitous prospective biofuel producers (Euglena) have unusual splice site and lack the canonical intron (GU-AG). This suggests the existence of an alternative mechanism of intron removal and implies diplonemids can be an excellent model for the study of intron evolution. We have therefore sequenced genomes and transcriptomes of several marine diplonemids. In this project, we will be carrying out comprehensive splice-site analysis of these species. Firstly, we will be annotating the genomes of all sequenced marine diplonemids, identify non-canonical introns and determine common sequence and/or structural features. Finally, we will perform comparative intron/splice site analysis among diplonemids, reveal general mechanisms and pattern of RNA splicing and compare it to the canonical eukaryotic model.

Examining factors mediating oligo uridylation and degradation of eukaryotic aberrant RNAs

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The 3`-end RNA uridylation has pivotal role in regulating the global gene expression in Eukaryotes. Terminal uridyltransferase catalyzes the additions of oligo uridine (U) addition to the precursors of different species of RNAs entering TUT-DIS3L2 pathway. DIS3L2 has a diverse RNA species substrate. Our lab identified DIS3L2 RNA substrates using CLIP-seq. TUT-DIS3L2 degradation pathway is not very clearly understood. To identify the co factors/RNA binding proteins which might be involved in this pathway, we have used proteomics-based pull down approach using RNA as a bait to map and characterize the interactome of TUT-DIS3L2 pathway factors. This RNA bait was chosen based on candidates having large number of reads from our CLIP-seq data. A complimentary biotinylated oligo to the bait was used to perform the experiments. This will help us to identify proteins which are involved in degrading the RNA species identified by DIS3L2 CLIP. Some preliminary proteins candidates have been identified which will be functionally validated.

Mechanisms of G-quadruplex biochemical specificity

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G-quadruplexes are noncanonical nucleic acid structures formed by stacked guanosine tetrads. They bind a variety of proteins and small molecules, catalyze several types of reactions, form intrinsically fluorescent structures, and are thought to play widespread biological roles. This diversity raises an important question: what determines the specificity of G-quadruplex biochemical function? This is particularly important from the perspective of biological regulation because eukaryotic genomes can contain hundreds of thousands of G-quadruplex structures. To address this question we analyzed the biochemical specificity of each sequence in a 496-member G-quadruplex library with respect to five biochemical functions: the ability to bind GTP, to promote peroxidase reactions, to generate fluorescence, to form dimers, and to form tetramers (1-4). Our analysis indicates that each of these functions can be distinguished from the others at the level of primary sequence, with some mutations altering G-quadruplex biochemical specificity by orders of magnitude. Mutations in tetrads tend to have larger effects than mutations in loops, and changes in specificity are correlated with changes in both the multimeric state of the G-quadruplex (5) and the structural context of terminal tetrads. Taken together, these results indicate that significant changes in the biochemical specificity of a G-quadruplex can be achieved by mutations in its primary sequence. They also highlight the roles played by multimerization and the structural context of flanking tetrads in modulating G-quaduplex biochemical specificity.

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Genome organization of T cells as a determinant of (auto)immunity

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Long-range chromatin interactions in eukaryotic genomes are reliant on the interplay of specific protein factors. This is now becoming appreciated as a potential epigenetic mechanism of cell memory and cell lineage specification. One such factor being highly expressed during T cell development and possibly organizing chromatin of T cells is the Special AT-rich sequence Binding protein 1 (SATB1). Here we present a comprehensive study of both structural and regulatory roles of SATB1 in the thymus and propose a mechanism for autoimmunity onset in *Satb1*fl/flCD4*Cre*+ mice.

In order to investigate the roles of SATB1, we created *Satb1*fl/flCD4*Cre*+ conditional knockout mice (*Satb1* cKO). These animals have a smaller thymus and evince a blockade at the CD4+CD8+ double positive (DP) stage. Absence of SATB1 results in global heterochromatinization of T cell nuclei, causing underexpression of many receptor genes responsible for T cell communication within the thymus (RNAseq, ATACseq).

Transmission electron microscopy experiments revealed that the lack of communication evolves in disrupted structure of the thymus. It further leads to impaired positive and negative selection of T cells. Altogether it allows some of the T cells to escape the thymus prematurely, which we detected as autoreactive T cells infiltrating peripheral organs.

Since SATB1 is considered to be a genome organizer, we employed HiChIP and HiC experiments to unravel its potential architectural role. We compared the SATB1-mediated chromatin loops with these mediated by the conventional organizer CTCF and the corresponding changes in the *Satb1* cKO thymocytes. Based on our data, we propose SATB1 looping to be a direct regulatory mechanism of T cell receptor recombination. Moreover, SATB1-mediated loops show a clear specificity towards immune-related genes. These loops have activatory characteristics and many of them connect genes with enhancers (confirmed also by H3K27ac HiChIP in WT and *Satb1* cKO cells). We demonstrated the importance of the SATB1-enhancer loops on the regulation of *Bcl6* gene, a master regulator of T cell development.

Moreover, we propose an explanation of higher prevalence of autoimmune diseases in female mice. Localization of SATB1 is clearly dependent on the presence of RNA.

We show that SATB1 binds Xist lncRNA and it is responsible for its spreading. This is a characteristic and most likely a vital feature of DP T cells which normally keep their inactivated X chromosome more euchromatinized. In *Satb1* cKO we demonstrated the lack of Xist spreading resulting in the enormous increase of DNA methylation on the X chromosome together with more inaccessible regions and loss of expression of escapee genes from the inactive X chromosome. X chromosome harbors many immune-related genes among which are included the important cell surface receptor *Cd40l* and the previously studied *Tlr7* which we both found to be significantly downregulated in the *Satb1* cKO. These, together with the deregulated TCR further contribute to the impaired T cell communication within thymus.

Collectively, our data clarify the CD4+ T cell genome organization and delineate role of SATB1 in T cell development and differentiation. Our results underline the contribution of T cell chromatin (de)regulation in the onset of autoimmune diseases and may further be utilized in designing targeted treatments.

The effect of the Perlman syndrome DIS3L2 exoribonuclease in the regulation of gene expression

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The 3'-terminal uridylation is catalyzed by the terminal uridyltransferases (TUTases). It is a widespread RNA modification mediating processing and/or degradation of various RNAs in most eukaryotes. Aberrant uridylated transcripts are specifically recognized by the Perlman syndrome exoribonuclease DIS3L2. In 2016 others and we demonstrated, that TUT-DIS3L2 (TDS) is a conserved RNA surveillance pathway in the cytoplasm [1-4]. TDS targets mostly aberrant noncoding RNAs. In addition, our studies uncovered uridylated transcripts corresponding to 5' termini of protein-coding genes (5' mRNA fragments, 5'mRFs). These fragments appear to originate from aberrant transcription initiation because the uridylation position overlaps with the position of stalled RNA Polymerase II.

In our follow up study, we investigate the process leading to the 5'mRFs formation, their uridylation dynamics and putative function in the cell. We are able to reconstitute their production from a heterologous reporter system. Our quantitative sequencing analyses show that 5'mRFs are formed in the nucleus. However, only after the export to the cytoplasm, they are targeted by the TDS. To test whether 5'mRF formation affects gene expression, we performed differential transcriptome-wide analysis of long and small RNAs from control and DIS3L2 K.O. cell lines. Next, we used the RNA-based immunoprecipitation to identify factors involved in the 5'mRF biogenesis and turnover. In summary, we will present a comprehensive analysis of the biogenesis and quality control of aberrantly terminated PolI transcripts in human cells.

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When Your Cell Culture Stares Back - An Emerging Model For Splicing-Associated Retinitis Pigmentosa

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Retinitis pigmentosa (RP) is a hereditary disease characterized by the progressive degeneration of retina cells eventually leading to total blindness of the patients. Currently more than 90 genes are known to be involved in the pathogenesis of RP. The majority of these genes is expressed in the retina and associated with retinal function. Surprisingly, the second largest group of mutations causing RP affects proteins involved in splicing. However, it remains elusive why mutations affecting RNA splicing, a ubiquitous and essential process for almost every cell, give rise to such a tissue-specific phenotype. To unravel the pathogenic mechanism underlying RP we are establishing a disease-relevant model. Using CRISPR/Cas9 genome-editing we introduce RP-associated mutations in splice factors in human induced pluripotent stem cells (hiPSC)-derived retina cells such as retinal pigment epithelium (RPE) or photoreceptor cells and retinal organoids. Here, we present and discuss preliminary results of our differentiation approaches of hiPSC to a pro-retinal phenotype and the development of retinal organoids. Our future goal is to determine the molecular mechanism by analyzing splicing efficiency, alternative splicing and alterations in interactions between RNA binding proteins and RNA using RNA-seq and iCLIP methods. Further, we want to study the effects of these splice factor mutations on morphology and function in RPE-, pohotorecptor cells and retinal organoids. The knowledge gained from our analysis will provide new insights into how splicing factor mutations are involved in the pathology of RP.

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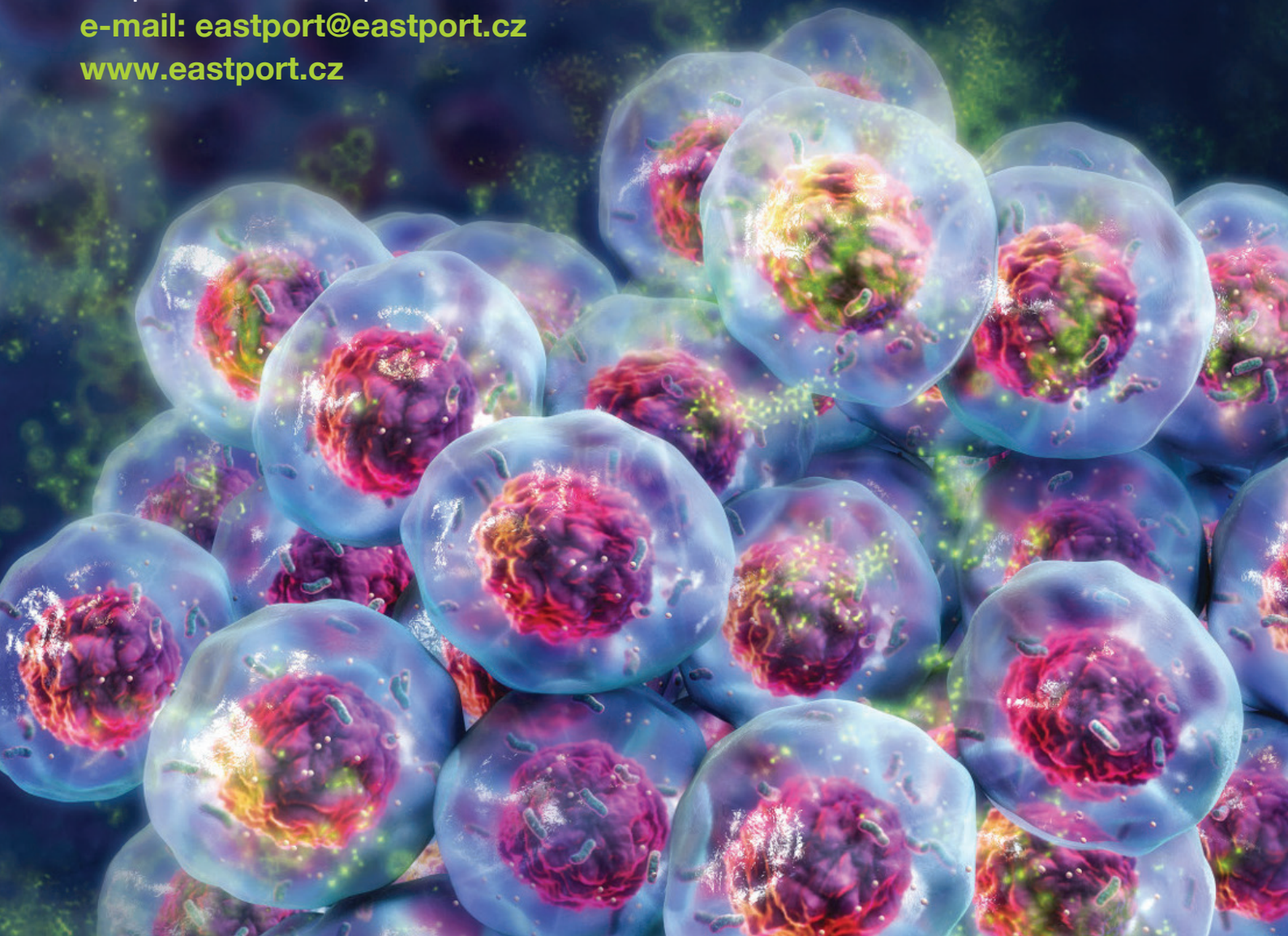

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Praktický workshop přípravy NGS knihovny LoopSeq™ 16S Microbiome SSC s vyhodnocením

Otestujte si pod odborným vedením vlastní metagenomické vzorky pomocí sekvenace nové generace.

V rámci workshopu si vyzkoušíte analýzu 16S rRNA ve směsných vzorcích pomocí unikátní technologie Loop Genomics (dlouhá syntetická čtení).

Součástí bude kompletní pracovní postup - laboratorní příprava NGS knihovny, vlastní sekvenování a následné bioinformatické vyhodnocení sekvenačních dat.

Co je náplní workshopu?

- Nabízíme jednodenní praktický workshop na přípravu NGS knihovny pomocí LoopSeq™ 16S Microbiome SSC 24-plex kitu včetně sekvenace na Ilumina NextSeq.
- Každý účastník bude mít v rámci workshopu možnost zpracovat tři vlastní vzorky.
- Kit umožňuje sekvenování všech variabilních úseků 16S rRNA v jednom čtení a umožňuje přesnou analýzu, identifikaci a kvantifikaci taxonů na základě sekvenování V1-V9 16S rRNA.
- Součástí bude také bioinformatické vyhodnocení sekvenačních dat pomocí cloud-based softwaru Loop Genomics.

Pro koho je workshop určený?

- Workshop je určený pro zájemce, kteří by si rádi vyzkoušeli přípravu a analýzu NGS knihovny pomocí LoopSeq™ 16S Microbiome z vlastních vzorků.
- Počet účastníku je maximálně 8.

Workshop bude probíhat: 10. 10. 2019

Kde: HPST, s.r.o., Na Jetelce 69/2, Praha 9 - Vysočany

Školitelé: Mgr. Petr Lněnička

Cena: 5.500,- Kč bez DPH

Registrace: e-mailem na **dgg@hpst.cz** (do předmětu prosím uveďte „Workshop – LoopGenomics)

Firma Loop Genomics nabízí unikátní systém přípravy NGS knihoven s tvorbou syntetických dlouhých čtení. Systém funguje na všech sekvenátorech Ilumina a to bez speciální instrumentace, jen s použitím běžného vybavení molekulárně biologické laboratoře.

Použitý molekulární barcoding slouží nejen ke složení dlouhého syntetického readu, ale i k významnému snížení chybovosti sekvenačních dat a zpřesnění kvantifikace.

Systém nabízí řadu aplikací:

- Metagenomika: full-lenght 16S/18S sekvenování, mykobiomika (18S + ITS1 a ITS2)
- Transkriptomika: full-lenght mRNA sekvenování
- DNA: de-novo sekvenování (scaffold), sekvenování long-range PCR amplikonů, klonální heterogenita a fázování záměn

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