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present

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RNAClub 2018

September 14, 2018, IMG CAS, Hašek lecture Hall, Prague, Czech Republic

PROGRAMME

08:00-09:00 **REGISTRATION**

09:00-09:10 **OFFICIAL OPENING**

Session I – Wild RNA things *chair: Hana Macíčková-Cahová*

09:10-09:40 **Edward Curtis** (IOCB ASCR)

Mechanisms of G-quadruplex biochemical specificity

09:40-10:00 **Eva Hegedűsová** (Paris lab, Biology Centre CAS)

Nuclear tRNA export in Trypanosoma brucei

10:00-10:20 **Jiří Pospíšil** (Krásný lab, IMB ASCR)

Bacterial nanotubes: morphology, genetic determinants and transfer of macromolecules

10:20-11:00 **COFFEE BREAK**

Session II – Lost in translation *chair: Leoš Valášek*

KEYNOTE LECTURE I

11:00-11:50 **Maria Hatzoglou** (Case Western Reserve University)

Surviving cellular stress via adaptive mRNA translation

11:50-12:10 **Jakub Zeman** (Valášek lab, IMB ASCR)

eIF3: A Structural Odyssey. An epic drama of adventure and exploration of yeast eIF3 structure

12:10-12:30 **Václav Vopálenský** (Pospíšek lab, CUNI)

Unconventional 5' and 3' UTRs of yeast linear plasmid mRNAs suggest a novel mechanism of translation

12:30-12:50 **Agnieszka Ciesielska** (Bio-Rad)

The unexplored transcriptome. Long noncoding RNA

12:50-15:00 <u>**LUNCH & POSTERS**</u>

Session III – From RBPs with love *chair: Petr Svoboda*

<u>KEYNOTE LECTURE II</u>

15:00-15:50 **Gunter Meister** (University of Regensburg)

Regulation of gene expression by RNA-binding proteins and non-coding RNAs

15:50-16:10 **Zuzana Krchňáková** (Staněk lab, IMG ASCR)

Polypyrimidine tract sequence determines splicing efficiencies of lncRNAs

16:10-16:30 **Tomasz Kabziński** (Štefl lab, MUNI-CEITEC)

Connecting 5' to 3' activities: Interaction between Rtt103p and Trf4p

16:30-16:50 **Dagmar Zigáčková** (Vaňáčová lab, MUNI-CEITEC)

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

16:50-17:20 <u>**COFFEE BREAK**</u>

Session IV – Nucleotides are forever *chair: Štěpánka Vaňáčová*

17:20-17:40 **Denisa Jansová** (Susor lab, IAPG)

RNA biosensors in living cells - Spatiotemporal mRNA translation

17:40-18:00 **Anna Šimonová** (Cahová lab, IOCB ASCR)

LC/MS analysis of RNA from viral particles

18:00-18:20 **Anzer Khan** (Keegan lab, MUNI-CEITEC)

Deciphering effects of Adar on Drosophila metamorphosis

18:20-18:50 **Juan Alfonzo** (Ohio State University)

Editing and modification by enzyme co-activation: A 10-year solution to a 25-year problem

18:50-19:00 **CLOSING REMARKS AND AWARDS**

19:00-24:00 **DINNER & PARTY**

LECTURES

LECTURE 1

Mechanisms of G-quadruplex biochemical specificity

Sofia Kolesnikova[1,2], Kateřina Švehlová[1,3], Tat'ána Majerová[1], Tereza Streckerová[1,2], Juan Alfonso Redondo Marín[1], Jan Šilhán[1], Václav Veverka[1,4], Edward Curtis[1]

1. Institute of Organic Chemistry and Biochemistry ASCR
2. Department of Biochemistry and Microbiology, University of Chemistry and Technology
3. Charles University in Prague, Faculty of Science
4. Department of Cell Biology, Charles University

G-quadruplexes are four-stranded nucleic acid structures thought to play widespread biological roles. The growing list of cellular processes thought to be regulated by DNA or RNA G-quadruplexes includes transcription, RNA processing, translation, and mRNA localization. A plethora of proteins have been identified that interact with G-quadruplexes in various ways, and handful of cellular cofactors that bind G-quadruplexes have also been identified. This diversity of biochemical function raises an important question: how does the cellular machinery distinguish the many G-quadruplexes in the genome? We are exploring the hypothesis that some of this specificity can be achieved by mutations in the primary sequence of the G-quadruplex itself. To test this idea, we generated a 496-member G-quadruplex library, and tested each member for five different biochemical activities associated with G-quadruplexes: the ability to bind GTP, to promote peroxidase reactions, to form dimers, to form tetramers, and to generate fluorescence. This revealed that mutations in both tetrads and loops can significantly alter the specificity of a G-quadruplex to favor a particular biochemical activity. In some cases, changes in specificity are correlated with changes in the multimeric state of the G-quadruplex. We also identified a small-molecule ligand that inhibits multimerization, raising the possibility that G-quadruplex specificity can be modulated by small molecules. We are currently using a combination of NMR and X-ray crystallography to better understand these mutations from a structural perspective, and preliminary results in this area will be discussed. Taken together, these experiments provide new information about the mechanisms of G-quadruplex specificity, and should facilitate analysis of the biochemical roles of these structures in cells.

LECTURE 2

Nuclear tRNA export in Trypanosoma brucei

Eva Hegedűsová[1,3], Alan Kessler[3], Sneha Kulkarni[1,2], Brandon Burgman[4], Juan Alfonzo[3], Zdeněk Paris[1,2]

1. Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic
2. Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
3. Department of Microbiology and The Center for RNA Biology, The Ohio State University, USA
4. The University of Arizona, Tucson, Arizona, USA

Protein and RNA transport across the nuclear envelope occurs through the nuclear pore complex (NPC) and requires proteins of the karyopherin family (exportins). Xpo-t in vertebrates and Los1 and Msn5 in yeast specifically export tRNAs from the nucleus. T. brucei is a single-cell parasite, 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. Our results indicate that similar to other eukaryotes, TbXpo-t is not essential for growth of trypanosomes. Moreover, RNAi silencing of TbXpo-t did not result either in disruption of the tRNA translocation, or in intron-tRNA accumulation in the nucleus, both phenotypes described for yeast mutants. These observations are discussed in the context of the possible roles of the mRNA export pathway in the translocation of mature tRNAs through the NPC and its role on tRNA processing.

LECTURE 3

Bacterial nanotubes: Morphology, Genetic determinants and Transfer of macromolecules

Jiri Pospisil[1], Oldrich Benada[2], Olga Kofronova[2], Michaela Sikova[1], Martin Hubalek[3], Hana Sanderova[1], Libor Krasny[1]

1. Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology of the CAS, Prague,
2. Laboratory of Molecular Structure Characterization, Institute of Microbiology of the CAS, Prague, Cz
3. Laboratory of Mass Spectrometry, Institute of Organic Chemistry and Biochemistry of the CAS, Prague,

Bacterial nanotubes (NTs) are membranous tubular structures. We study these structures in gram-positive bacterium *Bacillus subtilis*. The biogenesis, genetic determinants of NTs formation and their function are unknown. First, we characterized these enigmatic structures morphologically. In *B. Subtilis*, the NTs are typically formed at the cell poles and contain a specific terminal structure that we call "a flyswatter". Also, NTs may connect neighboroughing cells. Next, using a systematic screen, we identified a set of genes that are neccesseary for NT formation. Although NTs are formed relatively rarely, they offer frightening possibilities about material inter-cellular transfer. Indeed, we observed DNA transfer of non-conjugative plasmid DNA. To the contrary, we observed no protein or/and RNA transfer. Consistent with our genetic screen, mutants in genes identified as important for NT formation failed trasfer plasmid DNA. In summary, this study brings seminal information about (so far) poorly understood cellular structures that may change the view how microorganisms interact.

LECTURE 4

eIF3: A Structural Odyssey. An epic drama of adventure and exploration of yeast eIF3 structure

Jakub Zeman[1], Yuzuru Itoh[2], Michal Rosůlek[3], Zdeněk Kukačka[3], Daniel Kavan[3], Marat Yusupov[2], Yaser Hashem[4], Petr Novák[3], Leoš Shivaya Valášek[1]

1. Laboratory of Regulation of Gene Expression, Institute of Microbiology CAS
2. Institute of Genetics and Molecular and Cellular Biology, Illkirch, France
3. Laboratory of Structural Biology and Cell Signaling, Institute of Microbiology CAS
4. Institut Européen de Chimie et Biologie, Pessac, France

In eukaryotic translation, eukaryotic initiation factors (eIFs) are at least as important as the ribosome. Some of these factors play different roles throughout the entire process to ensure proper assembly of the preinitiation complex on the right mRNA, accurate selection of the initiation codon, errorless production of the encoded polypeptide and its proper termination. Perhaps, the most important one integrating signals from others and coordinating their functions on the ribosome is eIF3. In *S. cerevisiae*, eIF3 is formed by five subunits. Despite recent progress thanks to the use of a cryo-electron microscopy, its precise structure and position on 40S ribosomal subunit are still not known.

To crack mysteries of yeast eIF3, we used chemical cross-linking coupled to mass spectrometry, x-ray crystallography, and cryo-EM.

We demonstrate that eIF3 is very compactly packed when free in solution. This finding is in a sharp contrast with the situation when eIF3 binds 40S and embraces it almost completely. Our results also indicate that the association of eIF1 and eIF5 with eIF3 do not seem to dramatically change the globular shape of eIF3. Thus, we conclude that it is most probably the initial contact of eIF3 with the 40S that triggers the dramatic structural rearrangement of eIF3. In addition, using the same approach we have examined the so far unknown binding site of eIF5 on the 40S and propose how eIF5 influences binding of eIF3 on the surface of the 40S subunit.

Our study thus extends the knowledge of yeast eIF3, its geometry, and structural rearrangements provoked by its different binding partners.

LECTURE 5

Unconventional 5' and 3' UTRs of yeast linear plasmid mRNAs suggest a novel mechanism of translation

Vaclav Vopalensky[1], Michal Sykora[1], Tomas Masek[1], Martin Pospisek[1]

1. Laboratory of RNA Biochemistry, Department of Genetics and Microbiology, Charles University

Linear plasmids with almost identical compact genetic organization have been found in the cytoplasm of yeast species from nine genera. We employed pGKL1,2 plasmids from *Kluyveromyces lactis* as a model to investigate the previously unstudied transcriptome of yeast cytoplasmic linear plasmids. We performed 5' and 3' RACE analysis of all the pGKL1,2 mRNAs and found them not 3' polyadenylated and containing mostly uncapped 5' poly(A) leaders that are not complementary to the plasmid DNA. The degree of 5' capping and/or 5' polyadenylation is specific to each gene and is controlled by the corresponding promoter regions. We refined the description of the pGKL1,2 promoters and found new alternative promoters of several genes. We also provide evidence that K2ORF3 encodes an mRNA cap guanine-N7-methyltransferase and that 5' capped pGKL1,2 transcripts contain N7-methylated caps. Translation of pGKL1,2 transcripts is enhanced in lsm1 and pab1 strains and is independent of eIF4E and Pab1 translation factors. We suggested a model of a primitive regulation of pGKL1,2 plasmids gene expression where degree of 5' mRNA capping, degree of 5' non-template polyadenylation and presence of negative regulators as PAB1 and Lsm1 play an important role.

LECTURE 6

Polypyrimidine Tract Sequence Determines Splicing Efficiencies of Long Non-Coding RNAs

Zuzana Krchnakova[1], Prasoon Kumar Thakur[1], Michaela Krausova[1], Nicole Bieberstein[1], Michaela Muller-McNicoll[2], David Stanek[1]

1. Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
2. Institute for Cell Biology and Neuroscience, Goethe University, Frankfurt am Main, Germany

Many nascent long non-coding RNAs (lncRNAs) undergo the same maturation steps as pre-mRNAs of protein-coding genes (PCGs) including capping and polyadenylation, but they are often poorly spliced. To identify the underlying mechanisms for this phenomenon, we used a model lncRNA and showed that intronic sequences are primarily responsible for its inefficient splicing. Genome-wide analysis of intron splicing in intergenic lncRNAs (lincRNAs) using RNA-Seq data from five different human cell lines revealed that introns of efficiently spliced lincRNAs contain a higher thymidine content in the polypyrimidine tract (PPT) than inefficiently spliced lincRNAs and also efficiently spliced introns of PCGs. To test the role of the PPT experimentally, we raised the thymidine content in PPTs of six lincRNAs and observed enhanced splicing along with improved binding of the splicing factor U2AF2. Using iCLIP, we further found that lincRNA exons exhibit poor binding of the splicing enhancer proteins SRSF2, SRSF5 and SRSF6 compared to expression-matched PCG exons. We propose that lincRNAs lack the cooperative network of interactions that enhance splicing, which renders their splicing outcome more dependent on the optimality of PPT sequences and U2AF2 binding.

Connecting 5' to 3' activities: Interaction between Rtt103p and Trf4p

Tomasz Kabziński[1], Jana Laláková[2], Viacheslav Zemlianski[1], Karel Kubíček[1], Andrea Fořtová[1], Tomaš Klumpler[1], Štěpánka Vaňáčová[1], Richard Štefl[1]

1. Masaryk University
2. Karolinska Institute

RNA polymerase II (RNAPII) transcribes protein-coding mRNAs and a subset of non-coding RNAs (ncRNAs). For both, transcription termination is regulated by specific post-transcriptional modifications of C-terminal domain (CTD) of RNAPII. The aforementioned modifications are recognized by RNA processing and transcription termination factors via CTD-interacting domains (CIDs). Termination of ncRNAs is promoted by the NNS (Nrd1p-Nab3p-Sen1p) complex via the CID domain of Nrd1p. Nrd1p CID interconnects RNAPII termination with subsequent trimming by the nuclear exosome via the interaction with Trf4p subunit of the TRAMP (Trf4p-Air2p-Mtr4p) complex, an activator of the nuclear exosome. The CID of Nrd1p recognizes the C-terminal sequence of Trf4p that mimics phosphorylated CTD diheptad (Nrd1p-interacting motif; NIM) [1].

In this work, we found that Trf4p NIM interacts with mRNA termination factor, Rtt103p, via its CID. Interestingly, we found that Rtt103p interacts with an additional region of Trf4p termed RIM (for Rtt103p-interacting motif). By using NMR studies, we demonstrate that Rtt103p CID utilizes the same binding pocket to interact with CTD and both Trf4p sequences in a mutually exclusive manner. Furthermore, we show Rtt103p dimerization is crucial for binding with both interacting motifs of Trf4p.

In vivo, trf4 deletion strains show accumulation of extended, improperly terminated mRNAs. This phenotype is rescued by wild type, but not Trf4p defective in interaction with Rtt103p CID. RNAPII ChIP analysis on selected mRNAs did not reveal any substantial read-through phenotype. Rtt103p – Trf4p interaction, therefore, appears to be important for recruitment of the TRAMP complex and potentially exosome to proofread and remove improperly terminated mRNAs.

[1] Tudek, A., Porrua O., Kabzinski T. *et al.*; Molecular Cell (2014)

LECTURE 8

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

Dagmar Zigackova[1], Zuzana Feketova[1], Tomas Skalicky[1], Josef Pasulka[1], Mihaela Zavolan[2], Stepanka Vanacova[1]

1. Central European Institute of Technology, Masaryk University, Brno, Czech Republic
2. Biozentrum, University of Basel, Basel, Switzerland

The 3'-terminal RNA uridylation catalyzed by the terminal uridyltransferases (TUTases) mediates degradation of various RNAs and processing of some ncRNAs (1,2,3). DIS3L2 is mammalian oligo(U) specific exonuclease, that is involved in the decay of uridylated precursors of let-7 miRNAs, tRNAs, and cleaved mRNAs (4,5,6). Mutations in TUTases and DIS3L2 have been linked to cancer and developmental defects (7,8). We and others have recently demonstrated, that TUT-DIS3L2 (TDS) is a conserved cytoplasmic pathway for surveillance of aberrant transcripts (9,10,11,12). TDS targets mostly aberrant noncoding RNAs, such as snRNAs, rRNA, tRNAs, YRNAs, and also transcripts originating from pseudogenes. Interestingly, we also uncovered a fraction of transcripts corresponding to 5' termini of protein-coding genes (5' mRNA fragments, 5'mRFs).

In our follow up study, we investigate the process leading to 5'mRFs formation and uridylation. We could reconstitute the 5'mRFs formation from a heterologous reporter system. We show, that uridylated 5' fragments of mRNAs are exported to the cytoplasm, where they are removed by the activity of DIS3L2. However, the localization of the uridylation event and the proportion of uridylated vs. non-uridylated transcripts remained unclear. To address these questions we performed deep sequencing of specific RNA fragments from nuclear and cytoplasmic fractions. Thus together with our DIS3L2-Clip-seq and RNA-seq analysis we collected data elucidating the extent of TDS processivity and its impact on human transcriptome.

1. Lee M., et al. (2014), Cell, 2. Heo I., et al. (2009), Cell, 3. Heo I., et al. (2012), Cell, 4. Ustianenko D., et al. (2013), RNA, 5. Chang HM., et al. (2013), Nature, 6. Lubas M., et al. (2013), EMBO, 7. Astuti D., et al. (2012), Nat Genet, 8. Morris MR., et al. (2013), Am J Med Genet C Semin Med Genet, 9. Ustianenko D., et al. (2016), EMBO J, 10. Pirouz M., et al. (2016), Cell Rep, 11. Łabno A., et al. (2016), NAR, 12. Reimão-Pinto MM., et al. (2016), EMBO J

LECTURE 9

RNA biosensors in living cells -Spatio temporal mRNA translation in mitosis

Denisa Jansova[1], Daniel Mateju[2], Jeffrey Chao[2], Andrej Susor[1]

1. Institute of Animal Physiology and Genetics, ASCR, Rumburska 89, Libechov, Czech Republic
2. Friedrich Miescher Institute for Biomedical Research, Maullbeerstarsse 66, Basel, Swizerland

Gene expression includes mRNA biogenesis and its translation into protein on ribosomes. In recent years, a variety of techniques have been developed to measure translational and visualize the localization of translation in the cell. Temporal control of mRNA translation during the cell cycle is an important mechanism for regulating cellular processes. Mitosis is driven by precisely-timed changes in translation and protein degradation of specific proteins. Tanenbaum and others found a decrease of global translation in mitosis. For imaging of translation in live cell we used SunTag biosensor (Tanenbaum et al., 2014; Voigt et al. 2017), a reporter mRNA with fluorescently tagged RNA-binding proteins, allowing us a real-time imaging of nascent peptide synthesis from single mRNA in living cells. SunTag peptides provide multiple high-affinity binding sites for a single-chain variable fragment (SCFV) fused to GFP (Tanenbaum et al., 2014). The accumulation of many nascent polypeptide at sites of protein synthesis allows translation to be visualized. With translational reporter we focused on detection of the temporal and spatial regulation of translation during interphase and M-phase of the cell cycle of the live HeLa cells.

LECTURE 10

LC/MS analysis of RNA from viral particles

Anna Simonova[1], Barbora Svojanovska[1], Jan Paces[1], Martin Hubalek[1], Hana Cahova[1]

1. IOCB Prague, Flemingovo nam. 542/2, Praha 6, 16000

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, there are surprisingly scarce data on RNA modifications or RNA conjugates in any type of viruses. Development of vitally important methods for the sensitive analysis of RNA modification enabling detailed studies of the chemical structure of various RNA entities began only recently and they have never been applied to viral RNA. The simplicity of their genomes and well described structure and machinery of various viruses make them a perfect model system for searching for new RNA modifications as well as for understanding of the role of already known RNA modifications and RNA conjugates. In this particular project, we focus on HIV-1 (representative of *Retroviridae* family) and some eukaryotic viruses from order of *Picornavirales*. We isolated RNA from viral particles and analysed by LC/MS technique. The surprisingly high level of various methylations led us to the development of LC/MS quantification techniques. To reveal the exact position of methylation we had to apply the methylation profiling techniques in combination with next-generation sequencing. In next part of our studies, we will concentrate on understanding of the role of these methylated nucleotides in viral RNA.
Acknowledgment: The Ministry of Education, Youth and Sports supported this work from the programme ERC CZ (LL1603).

LECTURE 11

Deciphering effects of Adar on Drosophila metamorphosis.

Anzer Khan[1], Nagraj Sambrani[1], Barbora Novakova[1], Mary A. O'Connell [1], Liam P. Keegan[1]

1. CEITEC MU

One of the most prevalent type of RNA editing is the conversion of adenosine to inosine in double-stranded RNAs that is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. A→I RNA editing can lead to a codon change as the nucleoside inosine (I) is interpreted as guanosine (G) by ribosomes, resulting in a diversification of protein function. The ADAR family of proteins is present in all metazoans. In *Drosophila*, a single *Adar* is present on the distal X chromosome and is an orthologue of vertebrate *ADAR2.* In spite of major progress in the identification of editing sites, little is known about the regulatory mechanism of ADAR proteins in normal development and in disease. In this present study, we performed a genetic screen that has uncovered a novel effect of *Adar* on ecdysone signaling which is a crucial regulator of *Drosophila* development. Ubiquitous expression of *Adar* with the *act*5c-Gal4 driver results in pupal lethality with defects in ecdysis and head eversion. The lethality caused by ubiquitous expression of *Adar* can be rescued by blocking ecdysone synthesis and signaling. Tissue specific over-expression of *Adar* in the Prothoracic Gland (PG) with *phm*-Gal4 causes extended larval life with a long delay in pupation, with major reductions in prothoracic gland transcripts encoding the enzymes of ecdysone synthesis and signaling. These defects may be due to either aberrant RNA editing or RNA binding by ADAR protein. We hypothesize that *Adar* expression in *Drosophila* is a prerequisite to regulate ecdysone signaling during metamorphosis. Currently, we are dissecting regulation of the ecdysone pathway by *Adar* and pursuing loss of functions studies with *Adar* RNAi lines to decipher the role of *Adar* in metamorphosis of *Drosophila.*

POSTERS

POSTER 1

Molecular principles of Cajal body formation

Davide Alessandro Basello[1], Ivan Novotny[1], Michaela Efenberkova[1], Ales Benda[2], David Stanek[1]

1. Institute of Molecular Genetics, ASCR, Prague, Czech Republic
2. BIOCEV, Vestec, Czech Republic

The cell nucleus is a highly heterogeneous environment overcrowded of molecules. Part of the nuclear complexity rises from the presence of a number of different bodies, non-membrane bound structures, which accumulate various proteins and, often RNAs. The molecular principles behind bodies assembly and maintenance are recently a matter of an intensive debate. One of the "classical" examples of a nuclear body is the Cajal body (CB). CBs are involved in biogenesis, quality control and recycling of spliceosomal snRNPs. Coilin, the essential scaffolding protein of CBs, self-oligomerize and interacts with numerous proteins including snRNPs, and these interactions are important for CB formation. However, the basic information regarding its structure and function are lacking. Here, we test whether and how the interaction of snRNPs with coilin affects coilin self-oligomerization and CB formation. In our study we combine different live cell microscopy techniques together with molecular biology and biochemistry data in order to model CB assembly, maintenance and function.

POSTER 2

Retinitis pigmentosa linked mutation of Prpf8

Zuzana Cvačková[1], Michaela Efenberková[2], David Staněk[1]

1. Laboratory of RNA Biology, Institute of Molecular Genetics of the ASCR, Prague, Czech Rep.
2. Light Microscopy and Flow Cytometry, Institute of Molecular Genetics of the ASCR, Prague

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.
This work was supported by grant from GACR 18-01911J.

POSTER 3

RNA foci are present in DM1 patient cells but are undetectable in SCA3 and HD ones

Michal Michalak[1], Wlodzimierz Krzyzosiak[1], Karolina Drazkowska[1]

1. Institute of Bioorganic Chemistry Polish Academy of Sciences

DM1 (myotonic dystrophy 1), SCA3 (spinocerebellar ataxia 3) and HD (Huntington disease) are neurodegenerative disorders caused by the presence of abnormally expanded trinucleotide repeat tracts in certain genes. Since in SCA3 and HD repeat expansions consist of CAG triplets in ORFs, mutated proteins – ataxin 3 and huntingtin, respectively – contain long poly-glutamine (polyQ) tracts. On the contrary, CUG expansions characteristic to DM1 are present in the 3'UTR of DMPK transcript and thus have no impact on the amino acid sequence of the synthesized protein. Apparently, in DM1, the aberrant RNA molecule itself is the source of the problem – mutated transcripts are known to form nuclear foci, which sequester MBNL1 splicing factor, making the protein non-functional. RNAs containing both CUG and CAG tracts form hairpin structures in vitro, and there is a possibility they can do so in vivo as well, accumulating together with proteins bound to such structures. Here, we present the results of our investigation of RNA foci existence in patient fibroblast cells and model HeLa cells expressing transcripts with CUG or CAG tracts. To this end, RNA FISH experiments were performed, including use of the single molecule version of the method. We also immuno-stained poly-Q proteins searching for any aggregates in mutated cells. We do observe RNA foci In DM1 patient cells, but they are undetectable in fibroblasts from individuals affected with SCA3 and HD or in HeLa SCA3 model cell line. Immunostaining showed no clear differences between mutated and control cells.

POSTER 4

Looking for RPE ii. partner in GCN4 translation reinitiation

Stanislava Gunišová[1], Leoš Valášek[1]

1. Institute of Microbiology of the CAS, v. v. i., Videnska 1083, 14220 Prague, Czech Republic

Translation reinitiation (REI) is a gene-specific control mechanism characterized by the ability of some short upstream ORFs to prevent recycling of the post-termination 40S subunit in order to resume scanning for reinitiation downstream. Yeast *GCN4* mRNA contains four short uORFs in its 5’ leader, out of which the first two (uORF1 and uORF2) are highly REI permissive while the remaining two (uORF3 and uORF4) pose a barrier to *GCN4* translation. Previously we have demonstrated that the high REI competence of uORF1 and uORF2 depends on several *cis*-acting features out of which the REI-promoting elements (RPEs) create a specific structural arrangement (5’ enhancer) in their upstream regions. uORF1 utilizes four RPEs (i.–iv.), whereas uORF2 separately utilizes only a single RPE v. (similar in sequence with the uORF1-specific RPE i.) and, in addition, shares the function of RPE ii. with uORF1. We have also revealed that, in order to retain 40S subunit on mRNA upon termination, RPE i. and RPE iv. of uORF1 and RPE v. of uORF2 co-operate with the extreme N-terminal domain of the a/TIF32 subunit of the eukaryotic initiation factor 3 (eIF3). In our most recent work we provided direct *in vivo* evidence of these interactions and also proved the long-standing hypothesis that eIF3 is one of the initiation factors preserved on the 80S ribosomes during early elongation of translation, an essential precondition for its role in REI. However, the functions of remaining RPEs (RPE ii. and iii.) in REI are still not known. Here we concentrated on finding the interacting partner(s) of RPE ii., a small stem-loop structure which makes critically important contribution to REI promoted by both permissive uORFs. We used *in vitro* transcribed RNAs containing wild-type and mutated segments of *GCN4* 5’ enhancer as baits in RNA-protein pull-down experiments and assayed cellular extract prepared from *S. cerevisiae* under various conditions. The subsequent mass-spectrometry analysis revealed a number of potential RPE ii.-binding candidates with functional relation to translation machinery which we have started analyzing by genetic and biochemical testing. Determination of molecular roles of all functional elements regulating the *GCN4* expression will provide important information towards elucidation of the molecular mechanism of translational reinitiation the nature of which is largely still mysterious.

POSTER 5

Functional and biochemical characterization of human eIF3

Anna Herrmannová[1], Susan Wagner[1], Leoš Valášek[1]

1. Institute of Microbiology ASCR, Laboratory of Regulation of Gene Expression

The 12-subunit mammalian eIF3 is the largest and most complex translation initiation factor. It has been implicated in numerous steps of translation initiation, termination and ribosomal recycling. Eight subunits (a, c, e, f, h, k, l, m) form a structural scaffold, called PCI/MPN octamer. The remaining four non-octameric subunits (b, d, g and i) are most probably rather flexible. Imbalanced eIF3 expression levels are observed in various types of cancer and developmental disorders, but the consequences of altered eIF3 subunit expression on its overall structure and composition, and on translation in general, remain unclear. We presented the first comprehensive in vivo study monitoring the effects of RNAi knockdown of each subunit of human eIF3 on its function, subunit balance and integrity (Wagner et al. 2016). We showed that expression of eIF3 as a whole is a complex interconnected system – downregulation of one subunit decreases protein levels of other eIF3 subunits while their mRNA levels remain unchanged. Disrupting the octamer produces an array of different eIF3 subcomplexes. In our follow up study we investigate binding efficiency of these subcomplexes to 40S ribosomes and also their ability to recruit mRNA and thus produce functional pre-initiation complexes. For example, in the eIF3dKD the eIF3 complex lacking only the d subunit binds to 40S with strongly decreased efficiency suggesting, that eIF3d could be an important anchor point of eIF3 to the ribosome. On the other hand, in cells lacking eIF3k and l subunits (eIF3lKD), we observed increased recruitment of mRNA to 40S.

POSTER 6

Ms1 RNA increases the amount of RNA polymerase in Mycobacterium smegmatis

Jarmila Hnilicová[1], Michaela Šiková[1], Martina Janoušková[1], Olga Ramaniuk[1], Petra Páleníková[1], Martin Převorovský[2], Libor Krásný[1]

1. Department of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Rep.
2. Department of Genomics and Bioinformatics, Institute of Molecular Genetics, Czech Rep.

Ms1 is a sRNA recently found in mycobacteria and several other actinobacterial species. Ms1 interacts with the RNA polymerase (RNAP) core devoid of sigma factors, which differs from 6S RNA that binds to RNAP holoenzymes containing the primary sigma factor. Here, by RNA-seq we show that Ms1 is the most abundant non-rRNA transcript in stationary phase in *Mycobacterium smegmatis*. The accumulation of Ms1 stems from its increased synthesis combined with decreased degradation. We identify the Ms1 promoter, PMs1, and cis-acting elements important for its activity. Then, by comparing the transcriptomes of *wt* and *Ms1-null* strains from stationary phase, we reveal that Ms1 affects the intracellular level of RNAP. The absence of Ms1 results in decreased level of the mRNA encoding beta and beta´ subunits of RNAP, which is reflected also at the protein level. Thus, the *Ms1-null* strain has a smaller pool of available RNAPs that can be utilized when the transcriptional demand increases. This contributes to the inability of the *Ms1-null* strain to rapidly react to environmental changes during outgrowth from stationary phase.

POSTER 7

Deciphering the mechanics of translation control of ATF4 under stress

Vladislava Hronová[1], Leoš Shivaya Valášek[1]

1. Institute of Microbiology, AS CR, Prague

ATF4 (activating transcription factor 4) is the key player in the process called integrated stress response (ISR) which is an important cellular program enabling to manage incoming stress stimuli. It has been shown that translational upregulation of *ATF4* mRNA in stress is governed by a gene-specific regulatory mechanism called translation reinitiation. This process exploits the ability of short uORFs (upstream open reading frames) present in *ATF4* mRNA 5´UTR to retain the post-termination 40S ribosomal subunit on the mRNA. Both in yeast and humans it has been shown that reinitiation requires a presence of *cis*-acting sequences surrounding a reinitiation-permissive uORF that functionally interact with specific subunits of eIF3 (eukaryotic initiation factor 3). Establishment of this contact post-termination is crucial for stabilization of the small ribosomal 40S subunit on the mRNA and subsequent reinitiation. Despite the fact that uORF-driven *ATF4* expression serves as one of the textbook examples of translational control via reinitiation, there are still many open questions as how exactly it is regulated under various stresses. There are also many specific features within *ATF4* mRNA, which have never been investigated even though they may significantly contribute to the translation control of *ATF4* mRNA. Hence, we created a comprehensive battery of *ATF4* reporter plasmids and we tested them under normal and corresponding stress conditions to properly decipher all modes of translational control of *ATF4*.

POSTER 8

Synthesis and Degradation of Ms1 in Mycobacterium smegmatis

Martina Janoušková[1], Michaela Šiková[1], Jiří Pospíšil[1], Petra Páleníková[1], Pavel Bartl[2], Jarmila Hnilicová[1], Libor Krásný[1]

1. Department of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sc
2. Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague

Adaptation of microorganisms is necessary for their survival in changing environment. In this process, regulatory roles are played by small non-coding RNAs (sRNAs). Ms1 is an abundant sRNA (rivalling in amounts those of rRNA) found in *Mycobacterium smegmatis* and it has homologs in all mycobacteria including *Mycobacterium turberculosis*. Ms1 forms a complex with the RNA polymerase (RNAP) core and it is a pleiotropic regulator of gene expression, enhancing survival of the cell under various types of stress. Ms1 is highly expressed and stable in stationary phase and it is rapidly degraded when the cell is shifted into nutrient-rich medium. The accumulation of Ms1 in the cell depends both on its synthesis and degradation but the specific mechanisms involved are unknown. Here, we identify and characterize the Ms1 promoter, the dynamics of Ms1 expression, and reveal the presence of a transcription factor involved in regulation of its expression. Further, we identify an RNase, Polynucleotide phosphorylase (PNPase) to interact with Ms1. With recombinant PNPase we demonstrate that it is able to degrade Ms1 *in vitro* and identify Ms1 secondary structures that affect its stability. RNAseq data show that PNPase is expressed ~10x more in exponential than in stationary phase, inversely correlating with the accumulation dynamics of Ms1. In summary, we provide a comprehensive characterization of how the intracellular level of Ms1 is controlled, paving the way to potential future designs altering its expression in the case of pathogenic species.
This work is supported by grant 946216 from the Grant Agency of Charles University.

POSTER 9

U6-specific factor SART3 participates in biogenesis of Sm-class snRNPs

Klara Klimesova[1], David Stanek[1]

1. Department of RNA Biology, Institute of Molecular Genetics CAS, Prague, Czech Republic

Spliceosomal small nuclear RNPs are complexes containing snRNA, Sm or LSm protein ring and a set of proteins specific for each particle. U1, U2, U4 and U5 (so called Sm-class) snRNPs are assembled in a multi-step biogenesis pathway that involves nuclear and cytoplasmic stages and the final steps occur in a sub-nuclear compartment, the Cajal body. We recently showed that accessibility of the Sm ring is the critical factor for snRNP accumulation in Cajal bodies. However, the Sm-interacting partner that captures snRNPs in Cajal bodies has not been clearly identified. Our previous results revealed that the SART3 protein is important for accumulation of incomplete U4, U5 and U6 snRNPs in Cajal bodies, but the molecular mechanism of the SART3-dependent targeting of incomplete snRNPs to the Cajal body is elusive. SART3 was identified as a U6 snRNP interaction partner and a U4/U6 di-snRNP assembly factor. Here, we provide evidence that the N-terminal part of SART3 also interacts with mono-U4 and U2 snRNPs and binds specifically immature particles localized in Cajal bodies. In addition, our results suggest that SART3 associates with U2 snRNP via Sm proteins. We propose a model that the interaction between SART3 and Sm proteins is a part of a complex quality control mechanism that detects incomplete snRNPs and tethers them in Cajal bodies.

POSTER 10

RP-causative mutations in splicing factor Prpf8 abolish cerebellar granular cell layer

Michaela Krausova[1], Jan Prochazka[2], Michaela Muller McNicoll[3], Radislav Sedlacek[2], David Stanek[1]

1. Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic
2. Czech Centre for Phenogenomics, BIOCEV, Vestec, Czech Republic
3. Institute for Cell Biology and Neuroscience, Goethe University Frankfurt, Frankfurt am Main, Germany

Germline mutations in Prpf8 that constitutes an essential scaffolding protein to the pre-mRNA splicing machinery underlie development of retinitis pigmentosa (RP), a degenerative disease characterized by progressive loss of retinal photoreceptor cells. Given the eye-exclusive manifestation, the phenotype suggests that certain cell populations are particularly sensitive for proper spliceosome assembly and/or its outcomes. We utilized TALEN and CRISPR/Cas9 genome editing tools to establish three novel murine strains harboring RP-relevant Prpf8 mutations: Prpf8 Tyr2334Asn substitution (N), a Prpf8 ORF prolongation variant (Δ17) and moreover, a Prpf8 null allele (Δ366) that serves comparison with other presumptive PRPF8 loss-of-function pathological mutations. We challenged the N- and Δ17 alleles by crossbreeding to hemi- (Δ366/N; Δ366/Δ17) and homozygosity (N/N; Δ17/Δ17), all conditions are viable and the expression from mutant alleles reaches comparable levels to wild-type. In comparison, Prpf8 is vital to embryonic development (Δ366/Δ366) and in adulthood, hemizygosity (Δ366/wt) does not result in haploinsufficiency. The Prpf8 mutants display strong neuromotoric phenotype with atrophy of cerebellar granule cell layer and peripheral nerve axon degeneration that manifests in tremor, decreased activity and gait disturbances. Retinal structures, surprisingly, do not show onset of degenerative processes. In parallel, we examined transcriptome-wide RNA-binding profile of the PRPF8-Y2334N-eGFP variant by iCLIP approach and recorded overall decreased binding to both 5´-ss and 3´-ss. Together, we combine dedicated *in vivo* and *in vitro* tools to address how inappropriate splicing execution eventually corrupts the fitness of RP-stricken cell populations.

POSTER 11

Queuosine: The role of an essential tRNA modification in parasitic protist Trypanosoma brucei.

Sneha Kulkarni[1,2], Helmut Stanzl[1,2], Alan Kessler[3], Eva Hegedűsová[1], Juan D Alfonzo[3], Zdeněk Paris[1,2]

1. Institute of Parasitology, Biology Centre, 37005 České Budějovice (Budweis), Czech Republic
2. Faculty of Science, University of south Bohemia, České Budějovice (Budweis), Czech Republic
3. Department of Microbiology and The Center for RNA Biology, The Ohio State University, Columbus, OH

A general feature of tRNAs is a high number of nucleotide modifications that are introduced post-transcriptionally. Queuosine (Q), one of the most complex tRNA modifications, is found at the first position of the anticodon (wobble base) of several tRNAs. Despite its omnipresence in bacteria and eukaryotes, the function of Q is not completely clear, although it is speculated to affect the rate and fidelity of translation. As eukaryotes cannot synthesize queuine, they rely on their environment, diet, or gut microbiome, to obtain this micronutrient. In this study, we have used the protozoan parasite *Trypanosoma brucei,* the causative agent of sleeping sickness in humans, as a model for a comprehensive analysis of the tRNA guanine transglycosylase (TGT), the enzyme responsible for Q-tRNA formation. Unlike its bacterial counterpart, in most eukaryotes TGT predominantly functions as a heterodimer. Using bioinformatic approaches, we identified two TGT subunits in *T. brucei.* Interestingly, contrary to reports in higher eukaryotes, TbTGT1/2 heteromer is localized to the nucleus. In order to determine the functional significance of Q-tRNA modification in trypanosomes, we generated a gene knock-out of the TbTGT2 and performed additional phenotypic *in vivo* characterization directly in the bloodstream of the mammalian host with the goal to simulate actual conditions associated with parasite infection. After infecting mice with the mutant parasites, we observed that it takes significantly longer for the trypanosome cells to appear in the blood, and eventually to kill the animals, as compared to WT parasites. Our data suggests that queuosine plays an important physiological role during survival of the parasites inside the mammalian host and may be at the heart of virulence. Currently, we employ state-of-the-art technique of 'ribosome profiling' to further explore the role of this modification during translation in the bloodstream stage of *T. brucei.*

POSTER 12

A missense mutation in DDX38 that causes retinitis pigmentosa

Mina Obuca[1], David Stanek[1]

1. Institute of Molecular Genetics of the ASCR, v. v. i. Prague, Czech Republic

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 retinitis pigmentosa (RP), a major cause of blindness, is caused by mutations in six snRNP-specific proteins. Recently, 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 affects splicing efficiencies of several ubiquitously expressed and retina specific genes. In the next steps we aim to introduce the RP mutation into the DHX38 gene using CRISPR technique and test how it affects splicing and interaction with spliceosome.

POSTER 13

Detection and analysis of free circulating long RNAs in colorectal cancer

Pavel Ostasov[1], Pavel Pitule[1]

1. Biomedical Center, Faculty of Medicine in Pilsen, Charles University

RNA is not confined to individual cells, but as was discovered in recent years, it is also part of different types of extracellular vesicles secreted by cells including ectosomes, exosomes, oncosomes and others.
Thus, RNAs isolated from these extracellular vesicles could be used as proxies to determine states of cells in various healthy or diseased tissues. While the focus is mainly on small circulating RNAs like miRNAs or piwi-RNAs, which are the most abundant species in these vesicles and outside of cells in general, we decided to focus on long circulating RNA species like lncRNAs and mRNA. While all RNA species allow analysis of their expression or mutations, long RNAs also allow detection ofedits or splicing events. These might not only help to identify changes that are occurring during tumorigenesis but might also reveal mechanisms that direct RNAs in these secretion vesicles. In this work we analyzed publicly available sequencing data of exosomal RNAs[1] as well as our data from extracellular vesicles from plasma in order to detect differences in RNA splicing variants between healthy volunteers and colorectal cancer patients. Overall there were detectable changes in splicing variants between healthy donors. Interestingly both publicly available and our own data contained a high levels of intronic sequences. This work has been supported by the Charles University Research Fund (Progres Q39), by Charles University Research Centre program UNCE/MED/006 "University Center of Clinical and Experimental Liver Surgery" and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.
[1] Li, S., Li, Y., Chen, B., Zhao, J., Yu, S., Tang, Y., Huang, S. (2018). exoRBase: a database of circRNA, lncRNA and mRNA in human blood exosomes. Nucleic Acids Research, 46(D1), D106–D112. https://doi.org/10.1093/nar/gkx891

POSTER 14

Investigating the role of human eIF3c and eIF3d in translation initiation

Terezie Prilepskaja[1,2], Anna Herrmannová[1], Susan Wagner[1], Leoš Valášek[1]

1. Institute of Microbiology ASCR, Laboratory of Regulation of Gene Expression
2. Charles University, Faculty of Science, Department of Genetics and Microbiology

Eukaryotic translation initiation factor 3 is a multifactorial complex, which participates in numerous steps of protein translation, including translation initiation. Considering it´s breadth of impact, it is no wonder eIF3 is often find to be deregulated in many types of cancer. Mammalian eIF3 has 12 subunits, out of which 5 have homologs in yeast. Start codon selection is an important step in initiation of translation, as fidelity of AUG recognition has impact on proteome composition. The role of eIF3 in translation initiation was intensively studied in yeast but not in mammals and human. In this study, we focus on protein-protein interactions among individual subunits of human eIF3 and the role of subunits c, d and e in stringency of AUG recognition. It was shown previously that the accuracy of AUG recognition is influenced by eIF1 and eIF5. Here we reveal that among all eIF3 subunits, eIF3c is the main binding partner for eIF1 and eIF5 and we further specify the eIF1 and eIF5 binding sites on eIF3c. We examine the influence of eIF3c, d and e downregulation on stringency of start codon recognition and present an interaction map between eIF3d and eIF3 octamer and selected ribosomal proteins. Altogether, this study aims to illuminate the mechanism of function of eIF3c and eIF3d in start codon recognition and translation initiation.

POSTER 15

Regulatory network of Bacillus subtilis main and alternative σ factors during spore germination and

Olga Ramaniuk[1], Olatz Ruiz Larrabeiti[1], Martin Modrák[2], Libor Krásný[1], Jiří Vohradský[2]

1. Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sc
2. Laboratory of Bioinformatics, Institute of Microbiology, Czech Academy of Sciences

σ factor is an essential transcription initiation factor in bacteria. A fundamental task is identification of target genes of various σ factors. Each bacterial species has one primary and several alternative σ factors. Primary σ factor drives gene expression in a bacterial cell under favorable conditions, while alternative σ factors take control when diverse stresses come. In past years, there was a burst of sequencing data accumulation based on ChIP-seq and RNA-seq data. These approaches provide information on singular interactions between σ factor and its potential targets. Nevertheless, this information is fragmentary and does not provide comprehensive view of the gene regulatory network in the studied organism. A highly useful tool for discovering gene regulatory networks that complements sequencing approaches is kinetic modelling of gene expression.

Bacillus subtilis is a widely used Gram-positive model organism containing 18 σ factors. In our previous study we used data of gene expression profiles from 14 time points to model gene regulatory network of the primary σ factor – σA – in *B. subtilis* during spore germination and outgrowth, subsequently verifying selected predictions experimentally.

In this study we focused on the regulatory network of alternative *B. subtilis* σ factors during spore germination and outgrowth. We used kinetic modelling and meta-analysis to create regulatory network between alternative σ factors and their targets. For selected σ factors σB, σD, σH, σI, σM we set up *in vitro* transcription systems to verify predicted interactions experimentally. The outcome is a comprehensive overview of alternative σ factors regulatory network in *B. subtilis*.

POSTER 16

Quality control in snRNP biogenesis

Adriana Roithova[1,2], David Staněk[1]

1. Institute of molecular genetics ASCR, Prague
2. Faculty of Science, Charles University in Prague

Small nuclear ribonucleoprotein particles (snRNPs) are essential components of a spliceosome that catalyzes the crucial step of the gene expression called pre-mRNA splicing. snRNPs consist of small non-coding RNA, Sm proteins, which form the Sm ring around a specific Sm-binding sequence and a set of the proteins specific for each snRNP. snRNPs create the catalytic core of the spliceosome and therefore have to be under strict quality control. Previously, was shown that Xrn1 exonuclease degrades snRNAs lacking the Sm ring and they are accumulated in P bodies. However, the molecular mechanism how the cells discriminate between mature and immature particles is still mostly unknown. In this work, we focus on molecular mechanisms that detect defective snRNAs in the cytoplasm. We observed that LSm1 protein bind the snRNAs after disruption of the Sm ring assembly. Further, we depleted the SmB/B’ protein and observed localization of snRNPs in the P bodies, however, if we also depleted the LSm1 protein we lost the accumulation of defective snRNPs in P bodies. Together we found a new possible role of LSm1 protein in the localization of defective snRNPs in P bodies.

POSTER 17

Searching the promoter sequence determinant for NAD+initiation by RNA polymerase of Escherichia coli

Olatz Ruiz Larrabeiti[1], Daniel Pinkas[1], Natalya Panova[1], Libor Krásný[1]

1. Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic

The status of the 5' end of RNA affects its stability, localization and translation efficiency, and it is considered an epitranscriptomic regulatory element. The breakthrough discovery of the ubiquitous NAD+ redox cofactor attached to the 5'end of RNAs in prokaryotic and eukaryotic cells manifests the existence of a new cap-like RNA modification. Moreover, NAD+ capped transcripts were identified in *Escherichia coli*, revealing that this modification was mainly found in small regulatory noncoding RNAs, which have roles in bacterial adaptation and survival to adverse conditions. In the present, the biological significance of the NAD+ cap and its consequences for the RNA and the cell are unclear, but a number of mechanistical aspects of the NAD+ capping and its requirements are starting to be defined. For example, it has been showed that NAD+ can be used by RNA polymerase (RNAP) as a non-canonical transcription initiation nucleotide (NCIN) at promoters encoding +1A, and that the sequence of the promoter affects NAD+ capping efficiency. To further characterize this prokaryotic RNA capping, we focused on finding the promoter sequence determinants for NAD+ transcription initiation. For this, we created a series of chimeric promoters composed of the sequences of promoters that encode +1A and provide different NAD+ initiating efficiencies, and used them as templates in an *in vitro* transcription system. These experiments allowed us to measure the effect of the promoter element sequences on the NAD+ utilization efficiency by RNAP of *E. coli*. The results presented here constitute a step forward in the characterization of the mechanism of this newly found prokaryotic capping.

POSTER 18

HelD: Structure and Function

Petra Sudzinová[1], Tomáš Koval[2], Tomáš Kouba[3], Jan Dohnálek[2], Libor Krásný[1]

1. Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic
2. BIOCEV, Vestec near Prague, Czech Republic
3. EMBL Grenoble, 71 avenue des Martyrs, France

Previously, we identified a new interaction partner of RNAP in *Bacillus subtilis*, a helicase-like protein termed HelD (~ 90kDa). We showed that HelD binds to RNAP and stimulates its activity in an ATP-dependent manner by stimulating transcriptional cycling and elongation (Wiedermannova et al, 2014). But the specific role(s) and structure of HelD are still unknown.

Hence, we decided to solve the structure of HelD. First, we show here the shape of the protein by small angle X-ray scattering (SAXS) and we identify its approximate binding site on RNAP. We also demonstrate that the shape depends on ATP and the associated conformational change is then likely responsible for the observed ATP-dependent transcription enhancement.

Next, we present the structure of the RNAP:HelD complex from *Mycobacterium smegmatis* by cryogenic electron microscopy (cryoEM) at a resolution of ~3.8 Å. In the complex, HelD binds close to the primary channel of RNAP (consistent with the SAXS data) – the site of template DNA binding. This, then, has consequences for the stability of the elongation complex.
Finally, we reveal the importance of the unique N-terminal domain of HelD. This domain is not required for HelD binding to RNAP but is essential for its activity.

In summary, we provide detailed insights into the structure-function relationship of a novel binding partner of bacterial RNAP.

POSTER 19

Bioinformatics Analysis of Splicing Efficiency in Long Non-Coding RNAs

Prasoon Kumar Thakur[1], Zuzana Krchňáková[1], Michaela Krausová[1], Nicole Bieberstein[1], Michaela Müller-McNicoll[2], David Staněk [1]

1. Institute of Molecular Genetics of the ASCR, v. v. i, Prague, Czech Republic
2. Institute for Cell Biology and Neuroscience, Goethe University, Frankfurt am Main, Germany

Long non-coding RNAs (lncRNAs) undergo the same maturation steps as pre-mRNAs of Protein-coding genes (PCGs) including capping and polyadenylation, but they are often poorly spliced. Herein, we have computationally analyses the splicing efficiency of long intergenic non-coding RNAs (lincRNAs) and PCGs to understand the possible reasons of less-efficiently splicing of lincRNAs. Using iCLIP, we further compared the binding of SRSF2, SRSF5 and SRSF6 between PCGs and LincRNA. We propose that lincRNAs lack the cooperative network of interactions that enhance splicing, which renders their splicing outcome more dependent on the optimality of PPT sequences and U2AF2 binding.

POSTER 20

Functional analysis of the TSSC4 chaperone during snRNP formation

Jitka Vojáčková[1], David Staněk[1]

1. Department of RNA biology, Institute of Molecular Genetics ASCR, 142 20 Prague, Czech Republic

Splicing is a proces, during which non-coding introns are cleaved out of pre-mRNA and exons are ligated. This process is catalyzed by a multi-megadalton complex called spliceosome. The spliceosome is composed of five small nuclear ribonucleoproteins (snRNPs), which each contains a specific RNA molecule and a set of proteins. snRNPs undergo a complex assembly pathway, that includes cytoplasmatic and nuclear stage, during which the non-membrane nuclear organelles, Cajal bodies, are involved. Our previous mass spectrometry analysis have discovered several new proteins of unknown function in complex with U5 snRNP. The aim of this project is to analyze the function of one of them, namely TSSC4, to determine, whether the TSSC4 chaperone has a function during *de novo* assembly or recycling process of snRNPs and to discover the molecular function of TSSC4 in snRNP formation. Here we provide further evidence, that TSSC4 is interacting with U5 snRNP and we show the effect of TSSC4 knockdown in HeLa cells on the localization of snRNPs and a protein composition of U5 snRNP.

POSTER 21

Mapping of RNA/Protein Interaction Networks of Retinitis Pigmentosa Mutants

Felix Zimmann[1], David Staněk[1], Zuzana Cvačková[1]

1. Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

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 plan to establish a disease-relevant model. Therefore, we want to create RP-associated mutations in splicing factors through CRISPR/Cas9 genome-editing in human induced pluripotent stem cells (hiPSC)-derived retina cells such as retinal pigment epithelium (RPE) or photoreceptor cells. Here, we present a comparison of two protocols for differentiation of hiPSC to a pro-retinal phenotype. We will then analyze splicing efficiency, alternative splicing and alterations in interactions between RNA binding proteins and RNA using RNA-seq and iCLIP methods. The knowledge gained from our analysis will provide new insights into how splicing factor mutations are involved in the pathology of RP.

POSTER 22

Molecular aspects of aberrant CFTR exon 9 splicing orchestrated by TDP-43 and hnRNP A1

Maria Zlobina[1], Christelle Beaumont[1], Monika Kubíčková[1], Peter Josef Lukavsky[1]

1. CEITEC - Masaryk University, Brno, Czech Republic

The majority of human genes give rise to multiple protein isoforms with distinct functions through alternative splicing. Mutations in the pre-mRNA can alter the splicing outcome as in the case of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. TDP-43 binds with high affinity to a mutated, extended UG-rich region with a shortened polypyrimidine tract upstream of the 3’ splice site (3’ss) of CFTR exon 9 and recruits another splicing regulator, hnRNP A1. Thus formed complex prevents the recognition of the 3’ss of exon 9 by the spliceosomal machinery and causes skipping exon 9 resulting in a non-functional CFTR protein. Although RNA recognition and binding by TDP-43 alone and in complex with other hnRNPs has numerous functional implications, molecular details of such interactions remained elusive.

Our structural studies combined with biophysical approaches reveal that two copies of TDP-43 RBD create a new protein-protein interface with a salt bridge upon binding to the extended UG-rich sequence. Site-directed mutagenesis of amino acids involved in salt bridge formation reveals the functional significance of this protein-protein interface. Mutation at the interaction site of the two TDP-43 RBD copies reduces exon 9 skipping almost to the same extent as completely abolishing UG-rich RNA binding. This functional complex recruits hnRNPA1 to the intron 8 - exon 9 junction where it interacts with 3’ss RNA. NMR monitoring of the TDP-43 - hnRNPA1 complex assembly shows that their interaction with CFTR exon 9 3’ss RNA is not masking the polypyrimidine tract on the pre-mRNA for binding of the canonical splicing factor U2AF65. Shortening of the polypyrimidine tract, on the other hand, lowers the binding affinity of U2AF65 twofold. At the same time, hnRNPA1 binding at the 3’ss blocks access of U2AF35. Thus CFTR exon 9 skipping is driven by a network of interactions formed by TDP-43 and hnRNPA1 at the CFTR exon 9 3’ss which competes for the formation of the canonical splicing complex.

POSTER 23

Age related differences in the translational landscape of mammalian oocytes

Edgar del LLano[1], Tomas Masek[2], Lenka Gahurova[1], Marketa Koncicka[1], Anna Tetkova[1], Denisa Jansova[1], Martin Pospisek[2], Andrej Susor[1], Michal Kubelka[1]

1. Institute of Animal Physiology and Genetics
2. Department of Genetics and Microbiology, Faculty of Science, Charles University

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.

POSTER 24

Domain functions of Delta subunit of Bacillus subtilis RNA polymerase

Hana Šanderová[1], Alžbeta Rabatinová[1], Vojtěch Kubáň[2], Pavel Srb[2], Martin Blackledge[3], Tomáš Koval´[4], Lukáš Žídek[2], Libor Krásný[1]

1. Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology CAS
2. Central European Institute of Technology, Masaryk University, Brno, Czech Republic
3. University Grenoble Alps, CEA, CNRS, Institut de Biologie Structurale, Grenoble, France
4. Laboratory of Structure and Function of Biomolecules, Institute of Biotechnology CAS

Bacteria need to rapidly adapt to changing environment. One of the central enzymes for this process is RNA polymerase (RNAP). RNAP is an extensively studied multisubunit complex required for transcription of DNA into RNA. 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. 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 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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The Torpedo effect in Bacillus subtilis: RNase J1 resolves stalled transcription complexes

Michaela Šiková[1], Jana Wiedermannová [1], Martin Převorovský[2], Ivan Barvík[3], Petra Sudzinová[1], Oldřich Benada[1], Hana Šanderová[1], Ciarán Condon[4], Libor Krásný[1]

1. Institute of Microbiology, Academy of Sciences of the Czech Republic v.v.i., Videnska 1083, 142 20,
2. Faculty of Science, Department of Cell Biology, Charles University, Vinicna 7, 128 43, Prague 2, Cze
3. Faculty of Mathematics and Physics, Institute of Physics, Charles University, Ke Karlovu 5, Prague 2
4. UMR 8261 (CNRS—Univ. Paris Diderot, Sorbonne Paris Cité), Institut de Biologie Physico-Chimique, 13

RNase J1 is a unique bacterial exonuclease with 5' to 3' exonuclease activity recently discovered in *Bacillus subtilis*. We show here that *Bacillus subtilis* RNase J1 is able to resolve RNA polymerase complexes stalled on DNA. This resolution is mediated by the „torpedo“ mechanism where RNase J1 degrades the nascent RNA and upon collision with RNA polymerase causes the complex to dissociate. The absence of RNase J1 causes redistribution of RNA polymerase on DNA, increasing the amount of RNA polymerases per gene without a parallel increase in transcriptional output. In summary, we reveal here a novel molecular mechanism that ensures smooth functioning of the transcription machinery and contributes to the ability of the cell to survive.

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PARTICIPANTS

Kateřina Abrhámová
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
kari@natur.cuni.cz

Daria Aleshkina
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
aleshkina@iapg.cas.cz

Juan Alfonzo
The Ohio State University, 105 Biological Sciences Building, 484 West 12th Avenue, Columbus, OH 43210, United States
alfonzo.1@osu.edu

Martin Báječný
1st Medical Faculty, Charles University, U Nemocnice 5, Prague 2, 128 53, Czech Republic
pallbaid@gmail.com

Can Balaban
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
can-balaban@hotmail.com

Davide Basello
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
can-balaban@hotmail.com
basello@img.cas.cz

Roberto Benoni
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
roberto.benoni@uochb.cas.cz

Veronika Benson
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
benson@biomed.cas.cz

Michala Boudová
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
boudova.michala@gmail.com

Hana Cahova
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
cahova@uochb.cas.cz

Edward Curtis
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
curtis@uochb.cas.cz

Zuzana Cvačková
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
cvackova@gmail.com

Barbora Černá
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
cernaba3@natur.cuni.cz

Edgar del LLano
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
llano@iapg.cas.cz

Karolina Drazkowska
Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, Poznan, 61-704, Poland
reck.sc@gmail.com

Lucia Fehérová
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
lufeherova@gmail.com

Klára Frydrýšková
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
kazdovk@natur.cuni.cz

Elena Golovina
1st Medical Faculty, Charles University, U Nemocnice 5, Prague 2, 128 53, Czech Republic
elena.golovina@natur.cuni.cz

Stanislava Gunišová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
gunisova@biomed.cas.cz

Martina Hálová
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
martina.halova@natur.cuni.cz

Michael Hammond
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
mhammond@usc.edu.au

Hassan Hashimi
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
hassan@paru.cas.cz

Eva Hegedüsová
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
hegedusova.eva@gmail.com

Tomáš Heizer
1st Medical Faculty, Charles University, Kateřinská 32, Prague 2, 12108, Czech Republic
tomas.heizer@lf1.cuni.cz

Anna Herrmannová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
herrmannova@seznam.cz

Jarmila Hnilicova
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
hnilicova@biomed.cas.cz

Vladislava Hronová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
vlckovv1@natur.cuni.cz

Oldrich Hudecek
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
hudecek@uochb.cas.cz

Chia-Ling Chen
1st Medical Faculty, Charles University, Katerinska 32, Prague 2, 121 08, Czech Republic
charlene1989831@gmail.com

Martina Janoušková
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
martina.janouskova@biomed.cas.cz

Denisa Jansova
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
jansova@iapg.cas.cz

Tomasz Kabzinski
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
tomasz.kabzinski@ceitec.muni.cz

Shubhangini Kataruka
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
shubhangini.kataruka@gmail.com

Binnypreet Kaur
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic

binny@paru.cas.cz

Farkas Kerényi
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
farkas.kerenyi@biomed.cas.cz

Anzer Khan
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
anzer.khan@ceitec.muni.cz

Klara Klimesova
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
klara.klimesova@img.cas.cz

Markéta Končická
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
koncicka@iapg.cas.cz

Libor Krásný
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
krasny@biomed.cas.cz

Michaela Krausova
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
krausom@img.cas.cz

Zuzana Krchnakova
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
zuzana.krchnakova@img.cas.cz

Pavle Krsmanovic
1st Medical Faculty, Charles University, U Nemocnice 5, Prague 2, 128 53, Czech Republic
pavle.krsmanovic@lf1.cuni.cz

Monika Kubíčková
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
monika.kubickova@ceitec.muni.cz

Sneha Kulkarni
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
snehakulkarni.sk@gmail.com

Erika Kužmová
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
erika.kuzmova@uochb.cas.cz

Roman Leontovyč

Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
leontovyc.roman@seznam.cz

Jiří Libus
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
jiri.libus@natur.cuni.cz

Zuzana Loubalová
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
zuzana.loubalova@seznam.cz

Peter J Lukavsky
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
peter.lukavsky@ceitec.muni.cz

Tomáš Mašek
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
masek@natur.cuni.cz

Zora Melkova
1st Medical Faculty, Charles University, Studnickova 7, Prague 2, 128 00, Czech Republic
zmelk@LF1.cuni.cz

Mahabub Pasha Mohammad
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
mpasha@biomed.cas.cz

Eva Neuhöferová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
eva.neuhoferova@biomed.cas.cz

Mina Obuca
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
mina.obuca@img.cas.cz

Pavel Ostašov
Biomedical Center, Faculty of Medicine in Pilsen, UK, alej Svobody 76, Pilsen, 32300, Czech Republic
pavel.ostasov@lfp.cuni.cz

Mouna Ouchari
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
mouna.ouchari@uochb.cas.cz

Zdeněk Paris
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
parda@paru.cas.cz

Josef Pasulka
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
josef.pasulka@img.cas.cz

Jiri Pospisil
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
jiri.pospisil@biomed.cas.cz

Martin Pospíšek
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
martin@natur.cuni.cz

Klára Pospíšilová
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
pospisilova.klara@gmail.com

Terezie Prilepskaja
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
svoboter@natur.cuni.cz

Olga Ramaniuk
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
ramaniuk@biomed.cas.cz

Paul Reyes
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
gutierrez@uochb.cas.cz

Adriana Roithová
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
adriana.roithova@img.cas.cz

Kristina Roučová
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
ronea@seznam.cz

Olatz Ruiz Larrabeiti
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
olatz.rula@gmail.com

Martina Saláková
Faculty of Natural Sciences, Charles University, Průmyslová 595, Vestec, 25255, Czech Republic
martina.salakova@natur.cuni.cz

Karina Savvulidi Vargova
1st Medical Faculty, Charles University, U Nemocnice 5, Prague 2, 128 53, Czech Republic
vargova.mmbd@gmail.com
Marek Sebesta

CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
marek.sebesta@ceitec.muni.cz

David Stanek
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
stanek@img.cas.cz

Richard Stefl
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
richard.stefl@ceitec.muni.cz

Petra Sudzinová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
petra.sudzinova@biomed.cas.cz

Andrej Susor
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
susor@iapg.cas.cz

Petr Svoboda
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
svobodap@img.cas.cz

Eliska Svobodova
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
eliska1svobodova@gmail.com

Barbora Svojanovska
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
barbora.svojanovska@uochb.cas.cz

Michal Sýkora
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
michal.sykora@centrum.cz

Hana Šanderová
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
sanderova@biomed.cas.cz

Michaela Šiková
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
michaela.sikova@biomed.cas.cz

Anna Šimonová
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
a.simonova@uochb.cas.cz

Jana Šmahelová
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
jana.smahelova@natur.cuni.cz

Anna Tětková
Institute of Animal Physiology and Genetics CAS, Rumburská 89, Liběchov, 277 21, Czech Republic
tetkova@iapg.cas.cz

Prasoon Kumar Thakur
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
praasu@gmail.com

Pragya Tripathi
Biology Centre CAS, Institute of Parasitology, Branišovská 31, České Budějovice, 370 05, Czech Republic
tripathi.pragya540@gmail.com

Jana Trylcova
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
jana.trylcova@uochb.cas.cz

Lenka Ulrychová
Institute of Organic Chemistry and Biochemistry CAS, Flemingovo nám. 542/2, Prague 6, 16000, Czech Republic
lenka.ulrych@gmail.com

Leos Valasek
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic
valasekl@biomed.cas.cz

Stepanka Vanacova
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
stepanka.vanacova@ceitec.muni.cz

Jitka Vojáčková
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
jitka.vojackova@img.cas.cz

Vaclav Vopalensky
Faculty of Natural Sciences, Charles University, Viničná 5, Prague 2, 12843, Czech Republic
vasek@natur.cuni.cz

Oxana Yurikova
1st Medical Faculty, Charles University, U Nemocnice 5, Prague 2, 128 53, Czech Republic
oksasha1992@gmail.com

Jakub Zeman
Institute of Microbiology CAS, Videnska 1083, Prague 4, 142 20, Czech Republic

jakub.zeman@biomed.cas.cz

Dagmar Zigackova
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
zigackova.d@gmail.com

Felix Zimmann
Institute of Molecular Genetics CAS, Videnska 1083, Prague 4, 14200, Czech Republic
felix.zimmann@img.cas.cz

Maria Zlobina
CEITEC, Masaryk University, Kamenice 5, Brno, 63400, Czech Republic
maria.zlobina@ceitec.muni.cz

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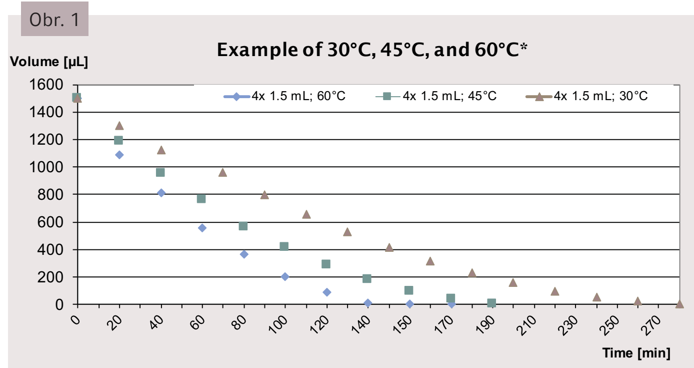

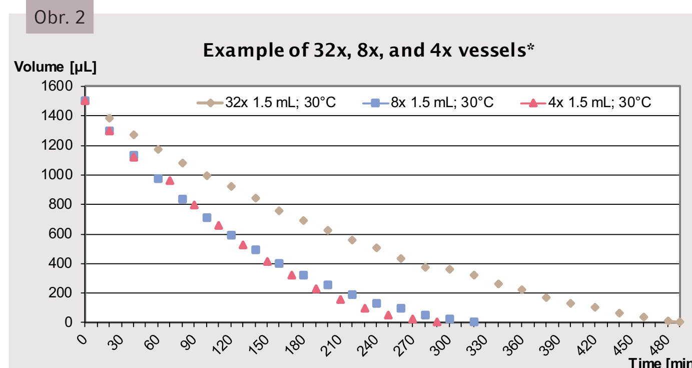

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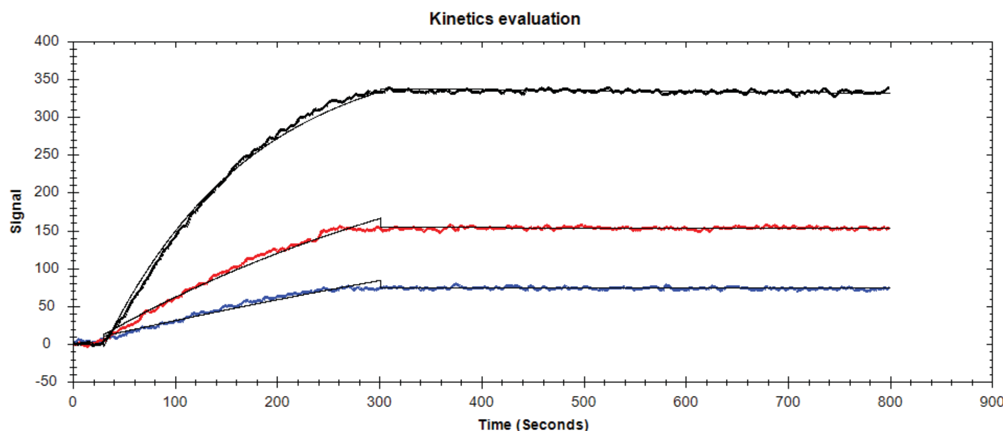

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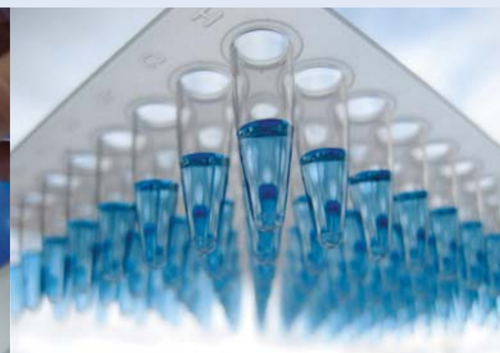

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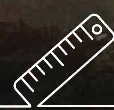

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The Invitrogen™ Qubit™ 4 Fluorometer and Qubit RNA IQ Assay offer the following features:

- **Convenience**—use as little as 1 µL of sample
- **Ease of use**—simply add RNA sample to the RNA IQ working solution, then measure with the Qubit 4 Fluorometer
- **Speed**—obtain accurate measurement of RNA degradation in ~4 seconds per sample

Don't forget that the new Qubit 4 Fluorometer also offers accurate and sensitive quantitation of DNA and protein.

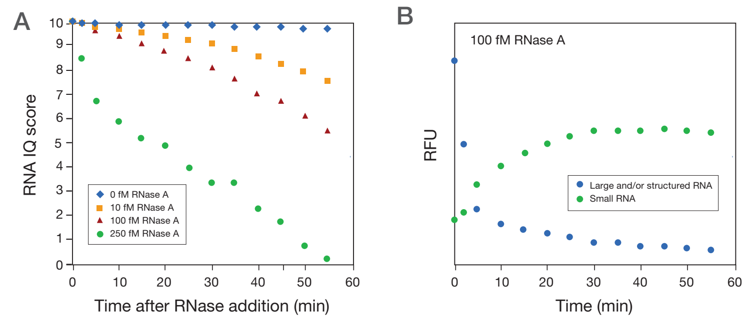

Figure 1. rRNA degradation by RNase A measured using the Qubit RNA IQ Assay. rRNA degradation by RNase A was measured in real time using the RNA IQ assay, demonstrating the loss of large, structured RNA and the increase of small, degraded RNA fragments over time.
(A) Triplicate samples of 100 ng/mL rRNA solutions were incubated with increasing amounts RNase A in the final RNA IQ assay working solution.
(B) The 100 fM RNase A shows the increase in signal from the small-RNA dye corresponding to a decrease in the signal from the large and/or structured RNA.

* Note: The Qubit RNA IQ Assay for the detection of degraded RNA can only be run on the Qubit 4 Fluorometer and cannot be performed on the original Qubit, Qubit 2.0, or Qubit 3.0 Fluorometers.

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