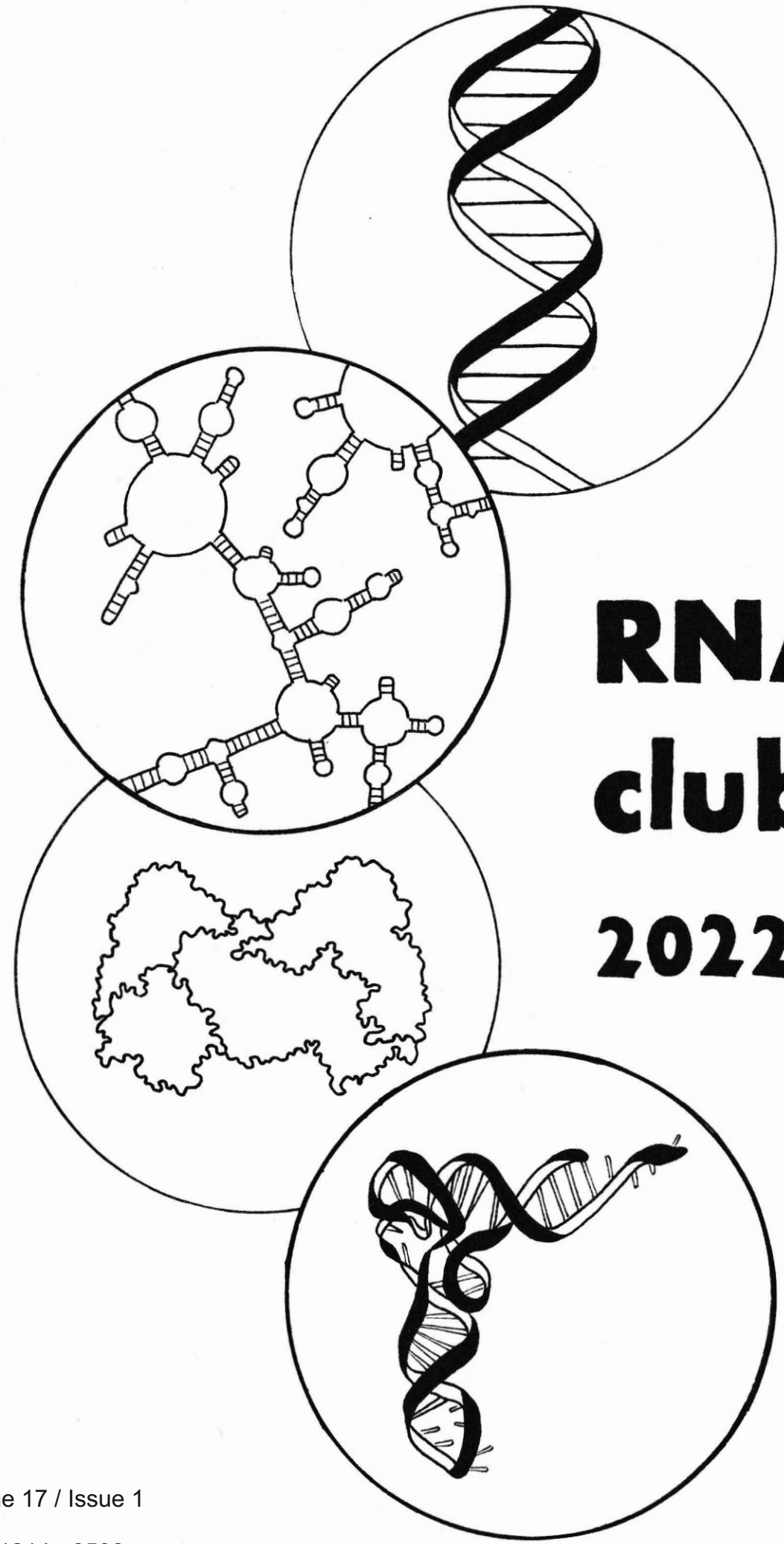

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RNA Club 2022

Prague, Czech Republic, October 3

PROGRAMME

8:30 – 9:40 **Registration**

Session 1: RNA modification

10:00 – 10:20 **Maria Bianca Mititelu**

Arabidopsis Thaliana Nudixes have RNA decapping activity

10:20 – 10:40 **Kristina Roučová**

Loss of ADAR1 protein induces changes in small RNA landscape in hepatocytes

10:40 – 11:00 **Veronika Rájecká**

An unbiased analysis of interplay between adenosine methylation and mRNA editing

Keynote lecture

11:00 – 11:50 **Finn Werner** (UCL; Structural and Molecular Biology)

The structural basis of RNA polymerase inhibition in response to virus infection

12:00 – 14:30 **Coffee / Lunch / Posters**

Session 2: Transcription and transcription regulation

14:30 – 14:50 **Petra Sudzinová**

Rifampicin, an inhibitor of RNA polymerase, can stimulate transcription

14:50 – 15:10 **Jana Dobrovolná**

DDX17 helicase mediates resolution of transcription-replication conflicts in human cells

15:10 – 15:30 **Hana Hanzlikova**

BRAT1 links Integrator and defective RNA processing with neurodegeneration

15:30 – 15:50 **Flash talks**

Tomáš Groušl

Xrn1 exoribonuclease – intrinsic marker for yeast population growth ("The mystery of P-bodies")

Jiří Pospíšil

σ*E of Streptomyces coelicolor can function both as an activator and repressor of transcription*

Jaclyn Quin

Targeting Adar1 in malaria

Nandan Varadarajan

Regulation of 5' mRNA fragments by TUT-DIS3L2 pathway and its association with Ago2

Diego Florián

Strategies for activating canonical RNAi in human cells

15:50 – 16:30 **Coffee Break / Posters**

Session 3: Ribosome biogenesis and translation

16:30 – 16:50 **Ondřej Gahura**

Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

16:50 – 17:10 **Anna Smirnova**

Ribosome queuing imposed by a stem-loop in the uORF2/ATF4 overlap region represents yet another level of the ATF4 translational control

Session 4: mRNA stability and processing

17:10 – 17:30 **Andrej Šušor**

CPEB3 makes a good egg

17:30 – 17:50 **Felix Zimmann**

Taking a closer look at the role of splicing factor mutations in retinitis pigmentosa using a retinal organoid disease model

18:00 **Conclusion**

18:00 – 19:00 Guided visit of the Church of the Assumption of the Virgin Mary and St. Charles the Great

19:00 – ??? **Dinner / Party** (U kalicha; Na Bojišti 12-14, 120 00 Praha 2)

LECTURES

Arabidopsis Thaliana Nudixes have RNA decapping activity

Mititelu M.B.(1), Hudeček O.(1), Gozdek A.(2), Nešuta O.(1), Kufel J.(2), Cahova H.(1)*

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Recently, new non-canonical RNA caps such as Nicotine Adenine Nucleotide (NAD)1, 2, 3'-dephospho-Coenzyme A (CoA)3 and dinucleoside polyphosphates (NpnNs)4 were discovered at the 5' end of RNAs in E. coli. NAD and CoA RNA caps were also found in other organisms e.g. in Arabidopsis thaliana5. These non-canonical RNA caps are cleaved from 5' RNA primarily by NudiX enzymes. Arabidopsis (Arabidopsis thaliana) contains 28 genes (AtNudt1–27 and AtDcp2) encoding NudiX hydrolase homologues6. In this work, we explore Arabidopsis thaliana NudiX enzymes and their substrate specificity. Particularly, we studied four enzymes: AtNudt6, AtNudt7, AtNudt19 and AtNudt27. As their substrate specificity on small molecules was known in vitro, we tested whether these enzymes are capable of cleaving these molecules once they are part of RNA, where they serve as 5' RNA caps. The identification of 5' capped RNA substrates for NudiX enzyme may give us hint what other RNA caps may exist in vivo in A. thaliana. Moreover, selective substrate specificity of some Nudixes may be useful tool in the development of techniques for identification of capped RNA sequences in combination with RNA-seq.

Loss of ADAR1 protein induces changes in small RNA landscape in hepatocytes

Roučová K.(1), Vopálenský V.(1), Mašek T.(1), del Llano E.(1,2), Provazník J.(3), Landry J.(3), Azevedo N.(3), Ehler E.(4), Beneš V.(3) and Pospíšek M.(1)

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In recent years, numerous evidence has been accumulated about the extent of A-to-I editing in human RNAs and the key role ADAR1 plays in the cellular editing machinery. It has been shown that A-to-I editing occurrence and frequency are tissue specific and essential for some tissue development, such as liver. To study the effect of ADAR1 function in hepatocytes, we have created Huh7.5 ADAR1 KO cell lines. Upon IFN treatment, the Huh7.5 ADAR1 KO cells show rapid arrest of growth and translation, from which they do not recover. We developed a new method for translatome analysis based on sequencing of separate polysome profile RNA fractions. We found significant changes in transcriptome and translatome of the Huh7.5 ADAR1 KO cells. The most prominent changes include negatively affected transcription by RNA polymerase III and the deregulation of snoRNA and Y RNA levels. Furthermore, we observed that ADAR1 KO polysomes are enriched in mRNAs coding for proteins pivotal in a wide range of biological processes such as RNA localization and RNA processing, whereas the unbound fraction is enriched mainly in mRNAs coding for ribosomal proteins and translational factors. This indicates that ADAR1 plays more relevant role in small RNA metabolism and ribosome biogenesis.

An unbiased analysis of interplay between adenosine methylation and mRNA editing

Rájecká V. (1), Sinigaglia K. (1), Stejskal S. (1), Covelo-Molares H. (1), Reyes Gutierrez P.E. (2), Macíčková Cahová H. (2), O'Connell M.A. (1), Vaňáčová Š. (1)

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(2) Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Prague, Czech Republic

The precise and unambiguous detection and quantification of internal RNA modifications represents a critical step for understanding their physiological function. Only handful of marks can be detected by reverse transcription and sequencing through the introduction of chemical conversions on isolated RNAs. Despite the methodological advances in the field, detection and quantification of m6A, m6Am and m1A modifications still remains one of the biggest challenges. In addition, it still remains obscure to which extent individual RNA modification marks are co-regulated or could potentially affect each other.
Here, we use an optimized quantitative approach to determine changes in steady state levels of m6A, m6Am and inosine upon impairment of METTL3, PCIF1, ALKBH5, FTO and ADAR1 activity in several human cell lines. Our results revealed that FTO depletion leads to elevated m6Am RNA levels in human cells, while depletion of ALKBH5 has only minor effects on m6A RNA levels. Remarkably, we show that upregulation of both FTO and ALKBH5 is accompanied by an increase of inosine steady-state level in mRNAs without affecting ADAR or dsRNA levels, thus supporting previous findings on negative correlation between adenosine methylation and A-to-I editing.

Rifampicin, an inhibitor of RNA polymerase, can stimulate transcription

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Rifampicin (RIF) is a potent inhibitor of bacterial RNA polymerase (RNAP) of clinical importance. It binds to RNAP and blocks transcription of RNA at the stage of 2-3 nucleotides made. Resistance to RIF can be mediated by mutations in its binding pocket on RNAP, by active efflux or enzymatic modification of RIF. Recent studies reported that HelD, an interaction partner of RNAP, contributes to rifampicin resistance in Actinobacteria but not in Bacilli.
Contrary to the published results, we noticed in our data that HelD expression in Bacillus subtilis was induced by RIF, suggesting its involvement in RIF resistance. Therefore, we initiated studies of B. subtilis HelD and showed that it increased the minimal inhibitory concentration (MIC), and its expression was stimulated by RIF at the transcriptional level. This was a conundrum as RIF is an inhibitor of transcription, and yet, it apparently stimulates it in the case of HelD. How is that possible? We, therefore, mapped the PhelD promoter and identified elements in the 5' UTR of the helD gene that are critical for the RIF-dependent stimulation of transcription. These elements are conserved in many Bacillus species.
The model of the transcriptional control of the B. subtilis helD gene by RIF will be thus presented and discussed.
This work was supported by grant No. 22-12023S from the Czech Science Foundation.

DDX17 helicase mediates resolution of transcription-replication conflicts in human cells

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R-loops are three-stranded nucleic acid structures composed of an RNA:DNA hybrid and displaced DNA strand, with a strong potential to halt DNA replication when formed co-transcriptionally in the opposite orientation to replication fork progression. Recent studies have shown that R-loop-stalled forks can be restarted by a mechanism involving fork cleavage by MUS81 endonuclease, reactivation of transcription and fork religation by the DNA ligase IV (LIG4)/XRCC4 complex. However, how R-loops are eliminated to allow for the sequential restart of transcription and replication in this pathway remains elusive. Here, we identified the human DDX17 helicase as a factor that associates with R-loops and counteracts R-loop-mediated replication stress to preserve genome stability. We show that DDX17 unwinds RNA:DNA hybrids in vitro and promotes MUS81-dependent restart of R-loop-stalled forks in human cells in a manner dependent on its helicase activity. Loss of DDX17 helicase induces accumulation of R-loops and the formation of R-loop-dependent anaphase bridges and micronuclei. These findings establish DDX17 as a component of the MUS81-LIG4 pathway for resolution of R-loop-mediated transcription-replication conflicts, which may be involved in R-loop unwinding.
This work was supported by the Czech Science Foundation (22-08294S and 21-22593X), the Czech Ministry of Education, Youth and Sports (LTAUSA19096) and by Charles University Grant Agency (1332217 and 308119).

BRAT1 links Integrator and defective RNA processing with neurodegeneration

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Mutations in BRAT1 have been associated with neurological disorders characterized by phenotypes with varying levels of clinical severity. However, the underlying molecular mechanisms of disease pathology remain poorly understood. Recently, we identified a novel homozygous missense c.185T>A variant in BRAT1 characterized by a much milder phenotype than previously reported, which greatly reduced the level of the BRAT1 protein in patient-derived cell lines. Surprisingly, our data show that this decreased level of BRAT1 does not interfere with ATM kinase activation or DNA damage response, suggesting that BRAT1 has another functional role, which if affected similarly leads to neurological disease. Excitingly, we identify BRAT1 as a novel binding partner of the catalytic RNA endonuclease heterodimer INTS9/INTS11 of Integrator complex. We show that insufficient and/or incorrect interaction of the BRAT1 protein with the INTS9/INST11 heterodimer impedes a processing of Integrator target RNAs. Importantly, Integrator dysfunction is also evident in cells obtained from BRAT1-related neurological disease patients. Taken together, our results support the model in which BRAT1 interacts with and stabilizes the INTS11, thereby maintaining the structural and functional integrity of the Integrator core cleavage heterodimer. Collectively, we suggest that defects in BRAT1 interfere with proper Integrator functions, leading to aberrant RNAs and proteins expression, resulting in neurodegeneration.

Biogenesis of trypanosomal mitoribosomes employs conserved and lineage-specific mechanisms and multitude of assembly factors

Gahura O., Chauhan P., Wong J.E., Dedkova J., Zikova A.

Mitoribosomes in trypanosomatid parasites diverged markedly from their bacterial ancestors and mitoribosomes in other eukaryotic lineages. We and others obtained cryoEM structures of several native precursors of large and small mitoribosomal subunits (mtLSU and mtSSU), which revealed that biogenesis of trypanosomatid mitoribosomes employ intricate machineries containing a multitude of assembly factors. We showed that the assembly of T. brucei mtSSU requires a homolog of RbfA, acting in biogenesis of bacterial ribosomes and mammalian mitoribosomes. Conserved core of TbRbfA binds key rRNA helices of the decoding center and mRNA channel and together with long lineage-specific extensions at both termini stabilizes the immature rRNA core. The C-terminal extension also serves as a binding platform for a heterodimer of two conserved methyltransferases and a kinetoplastid-specific pentameric assembly factor mt-SAF24. The NTD of mt-SAF24 forms a protrusion on the prospective intersubunit interface, which is capped by a disc-shaped density of unknown composition. We test the hypothesis that the density represents a piece of phospholipid bilayer and that the biogenesis of mtSSU occurs in the association with the inner mitochondrial membrane. Comparison of the trypanosomal pre-mtSSU complexes with a recently reported series of mammalian mtSSU precursors revealed common assembly mechanisms and allowed us to infer a chimeric pathway for mtSSU assembly.

Ribosome queuing imposed by a stem-loop in the uORF2/ATF4 overlap region represents yet another level of the ATF4 translational control

Anna Smirnova, Vladislava Hronová, Stanislava Gunišová, Pasha Mahabub Mohammad, and Leoš Shivaya Valášek

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ATF4 (Activating Transcription Factor 4) is a key player in the integrated stress response (ISR), an adaptive pathway enabling cells to respond to various stress stimuli. The ATF4 mRNA becomes upregulated under stress via delayed translation reinitiation (REI) mechanism, which exploits the ability of short upstream open reading frames (uORFs) to retain the post-termination 40S ribosomal subunit on the mRNA to allow REI downstream. The 5´UTR of ATF4 mRNA contains three uORFs (0 – 2), where uORF2 overlaps the main ATF4 ORF. Even though the original model of the ATF4 translational control was described for the first time in 2004, several critical discrepancies of it have remained unnoticed or at the minimum ignored. Therefore, using a large set of the ATF4-HA reporter constructs bearing various mutations of the human ATF4 mRNA-specific features (near-cognates, secondary structures and methylation motifs), we subjected the ATF4 translation control model to systematic re-analysis. We observed that, in contrast to the original model, uORF2 is robustly translated even under stress conditions. In fact, we discovered an existence of a semi-stable stem-loop in the uORF2/ATF4 overlap region that might act as a road block for ribosomes inducing ribosome queueing, and thus allowing uORF2 translation even under stress conditions. Therefore, besides delayed REI, ribosome queuing may play an equally important role in translational control of the ATF4 expression, at least in human cells.

CPEB3 makes a good egg

Lamacova L. (1), Iyyappan R. (1), Jansova D. (1), Dvoran M. (1), Fan H.Y. (2), Yuxuan J. (2), Masek T. (3), Pospisek M. (3), Susor A. (3)

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Controlled translation of maternal transcripts is required to coordinate mammalian oocyte meiosis and early embryo development upon ceased transcription. In particular, mRNA translation is regulated through many RNA-binding proteins. Here we show that the absence of polyadenylation element-binding protein 3 (CPEB3) negatively influences female reproductive fitness via early embryonic arrest. CPEB3 deficient oocytes progress normally through meiosis, however the aberrant transcriptome results in defects of protein expression and initiation of embryonic transcription. We found that CPEB3 stabilizes a subset of mRNAs with significantly longer 3'UTRs, enriched with cytoplasmic polyadenylation elements at their distal regions. Together, these findings reveal a key maternal factor regulating stability and translation of subclass of mRNAs essential for initiation of embryonic transcription and subsequent embryo development.

Taking a closer look at the role of splicing factor mutations in retinitis pigmentosa using a retinal organoid disease model

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Retinitis pigmentosa (RP) is the most common cause of inherited blindness worldwide. It is characterized by a progressive loss of photoreceptors and shows remarkable clinical and genetic heterogeneity. While most mutations causing RP have been found in proteins essential for photoreceptor function some mutations are surprisingly affecting ubiquitously required splicing factors such as Prpf8. The mechanism how these mutations cause RP remains elusive. Here we present the development of a human retinal organoid model to shed light on the underlying disease mechanism and the question of how mutations in splicing factors have such a retina-exclusive phenotype. Using CRISPR-Cas9 gene editing we introduced an RP-linked mutation into the regulatory Jab1/MPN domain of Prpf8 in human induced pluripotent stem cells (hiPSC) and differentiated them into three-dimensional retinal organoids. Our results show that retinal organoids differentiated from mutated hiPSC look-alike organoids from wild-type hiPSC containing both rod and cone photoreceptors as well as Müller and amacrine cells. However, transcriptome analysis revealed differential splicing of neural and retinal disease-associated genes as well as changes in expression of circular RNAs, while differential gene expression was only slightly affected. These data indicate that perturbation in splicing of specific genes and changes in expression of circular RNAs is causing the RP phenotype.

FLASH TALKS

Xrn1 exoribonuclease – intrinsic marker for yeast population growth ("The mystery of P-bodies")

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Xrn1 exoribonuclease is the major mRNA degradation enzyme of S.cerevisiae. Besides its localization through yeast cell, Xrn1 is gradually being sequestered into P-bodies as yeast population ages. P-bodies are RNA granules which were considered to be sites of the degradation of mRNA molecules. However, recent studies have challenged this hypothesis. We found that Xrn1 is localized also to the MCC/eisosome compartment at yeast plasma membrane [1]. This localization correlates with metabolic (diauxic) switch from glucose fermentation to respiration. Since this unique localization pattern, Xrn1 can be used as an intrinsic marker for yeast population growth [2]. At the same time, Xrn1 localization outside of P-bodies in post-diauxic growth phase supports rather cytoprotective yet still not completely elucidated function of P-bodies in cellular metabolism.

[1] Grousl T.*, Opekarova M.*, Stradalova V., Hasek J., Malinsky J., "Evolutionarily conserved 5'-3' exoribonuclease Xrn1 accumulates at plasma membrane-associated eisosomes in post-diauxic yeast", PLoS One, 2015. *These authors contributed equally to this work.

[2] Grousl T. et al., in preparation

σE of Streptomyces coelicolor can function both as an activator and repressor of transcription

Pospíšil J.(1), Schwarz M.(2), Ziková A.(2) Vítovská D.(1), Hradilová M.(3), Kolář M.(3), Krásný M.(1), Vohradský J.(2)

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Using a ChIP-seq approach, transcriptomic gene expression time series, promoter sequence analysis and computational modeling of gene expression we analyzed regulatory activity of an ECF sigma factor, σE, of Streptomyces coelicolor grown under standard conditions or induced by EtOH stress. We identified 91 candidate σE-controlled genes, of which 11 were exclusively found in the EtOH treated set of experiments. Modeling of gene expression using the time series measured under standard growth conditions then revealed that of the genes found by ChIP-seq, 28 genes (28%) were exclusively controlled by σE, 15 (15%) were found to be controlled by HrdB (the primary □ factor) instead of σE, and 48 (48%) had flat and/or low expression profile. Importantly, the computational and promoter sequence analyses showed that σE may act not only as an activator of gene expression but also as a repressor or act together with other sigma factors. The repressor function of σE was then confirmed by experimental analysis of selected promoter regions under normal and stress conditions. The results thus led to a new definition of the regulatory activity of σE in Streptomyces and suggest that it may be common for other factors and organisms

Targeting Adar1 in malaria

Quin J.(1), Arama C., Nébié I., Kopel E., Melicherová1 J., Musilová1 P., Sinigaglia K., Vukić D., Troye-Blomberg M., Sverremark-Ekström E., Modry D., Levanon E., Östlund Farrants A., Keegan L., O'Connell M.

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Adenosine-to-Inosine deamination of RNA by ADAR1 is essential to prevent activation of innate immune pathways by 'self' dsRNAs. Particularly, via activation of cytosolic dsRNA sensors including RLRs, PKR, and OAS-RNAse L. However, whether ADAR1 can be targeted to provide protection from infectious diseases is less clear.
In malaria studies, the Fulani ethnic group are protected from P.falciparum malaria. We observed reduced rates of A-to-I RNA editing in CD14+ monocyte innate immune cells following P.falciparum infection in the Fulani compared to a control unprotected ethnic group. Therefore, we have investigated whether reduction in A-to-I RNA editing can confer protection against malaria.
Analysis of publicly available RNA-sequencing data show transient but significant changes in levels of A-to-I RNA editing in individuals following Plasmodium infection, with reduced A-to-I RNA editing levels associated with protection from malaria. In addition, in vitro models of malaria infection utilizing cells isolated from malaria naïve blood donors display a rapid reduction in levels of A-to-I RNA editing in specific innate immune cell types. Finally, and most strikingly, Adar1+/- heterozygous mutant mice are protected from malaria, with significantly reduced parasitemia during blood stage infection with rodent malaria parasite P.yoelii.
Collectively our data support a model where reduced levels of ADAR1 activity contribute to protection from parasitemia during malaria.

Regulation of 5' mRNA fragments by TUT-DIS3L2 pathway and its association with Ago2

Nandan Mysore Varadarajan1,2*, Dagmar Zigackova1* Vaclav Hejret1, Praveenkumar Rengaraj1,2, Ales Obrdlik1, Panagiotis Alexiou1 , Stepanka Vanacova1

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RNA degradation and quality control in mammalian cells have several different mechanisms. In the cytoplasm, the uridylation-mediated DIS3L2 surveillance (TDS) targets a wide range of aberrant RNAs. Our previous analysis showed that TDS targets small RNAs including short transcripts originating from 5' ends of protein-coding transcripts (5'mRFs). Previously, a subset of these mRFs has been observed to give rise to functional miRNAs. It is yet to be established how these mRFs are regulated. We used one of the most abundant DIS3L2 targeted mRFs as bait to identify additional factors of the 5' mRFs biogenesis. The identified proteins included several factors of the RNAi pathway and other RNA binding proteins. This gave us the support of 5' mRF involvement in RNAi. Thus, we employed AGO2 CLASH analysis to identify putative mRNA targets of mRF-derived miRNAs. We found AGO2-bound chimeras of mRF-derived miRNAs and different mRNAs, which points toward their potential role in gene expression and regulation.

Strategies for activating canonical RNAi in human cells

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RNA interference (RNAi), sequence-specific mRNA degradation induced by double-stranded RNA (dsRNA), is the main antiviral innate immunity pathway in plants and invertebrates. In mammals, however, this pathway has been replaced by the interferon response, which relies on a set of protein sensors triggered by pathogen-associated molecular patterns. Endogenous full-length Dicer does not support RNAi well because it poorly processes dsRNA. In this project, we aim to boost RNAi in human cells by producing truncated Dicer variants which would have enhanced dsRNA-processing activity. For this purpose, we produced and tested truncated Dicer variants carrying deletions in the N-terminal helicase domain, which consists of HEL1, HEL2i and HEL2 subdomains. Up to this point, a combined △HEL1△HEL2 mutant is the most efficient RNA-inducing variant. Additionally, we tested Morpholino antisense oligos to induce exon skipping and produce a shorter Dicer variant from endogenously expressed Dicer. We report that it is possible to modify endogenous Dicer to enhance RNAi activity in human cells by altering splicing of endogenous nascent transcripts.

POSTERS

Novel mechanisms of rifampicin resistance in Bacillus subtilis

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(2) Mass Spectrometry Group, Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00, Prague 6, Czech Republic

Nowadays, antibiotic resistance is a serious global problem. Rifampicin is a clinically important antibiotic that acts against bacterial infections. Rifampicin binds to RNA polymerase (RNAP) and stops RNA synthesis at an early stage when only several nucleotides are transcribed. We were interested in novel mechanisms of rifampicin resistance in Bacillus subtilis. First, by a proteomic approach we identified proteins with altered levels after rifampicin treatment compared to an untreated sample. Subsequently, we focused on upregulated proteins as they could play roles in rifampicin resistance. Second, we examined by RT-qPCR whether the changes in protein levels were at the transcriptional or translational level. Third, we performed phenotypic assays, challenging knock-outs in genes encoding the upregulated proteins with sub-inhibitory concentration of rifampicin. Some of the knock-outs became more sensitive to rifampicin. The roles of selected rifampicin-upregulated proteins in rifampicin resistance as well as regulation of their gene expression will be presented and discussed.

Role of circular RNA in Retinitis Pigmentosa associated mutation

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Retinitis Pigmentosa(RP) is a genetic disorder causing the loss of photoreceptors and a corresponding loss in central and peripheral vision. Pre-ribosomal processing factor 8 (Prpf8) is a spliceosomal protein, mutation of which in humans results in an autosomal dominant form of RP. A mouse model having a missense mutation in Tyr2334Asn (Prpf8 Y2334N) was established in the lab phenocopying the human Prpf8 RP. Homozygous (N/N) mutants showed a pronounced phenotype in the cerebellum with a loss of granular cells. Deep RNA sequencing performed to analyze the transcriptome of Prpf8 N/N mouse cerebellum showed differential expression of a large number of circular RNAs (circRNA). Hence, we investigated circRNA biogenesis in the context of mutated Prpf8. We generated cerebellum-specific circRNA reporters and monitored circRNA expression in the wild-type (WT) and N/N hRPE cell lines by RT-qPCR. We detected a differential expression of some circRNA reporters in RPE consistent with RNA-seq results in the cerebellum showing that Prpf8 mutation affects circRNA biogenesis. We further studied circRNAs expression in human retinal organoids and showed their differential expression between wt and N/N organoids by RT-qPCR. We also found significant variability in circRNA expression in different human cell lines demonstrating their tissue specificity.

Identification of novel interacting partners of RNA polymerase in Mycobacterium smegmatis

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Transcription is a crucial process of gene expression that requires coordinated interaction of DNA-dependent RNA polymerase (RNAP) with several other transcription factors. Our knowledge of the bacterial transcriptional machinery largely comes from the established bacterial model systems such as Escherichia coli and Bacillus subtilis. However, studies have shown that different bacterial species deploy discrete transcription factors to optimally regulate gene expression. In this regard, Mycobacterium smegmatis has evolved as another model organism to study the transcription factors in prokaryotes. Discoveries of two essential mycobacterial transcription factors- CarD and RbpA, absent in E.coli, have encouraged researchers to search for novel transcription factors that would help understand the complexity and versatility of bacterial gene regulation. In this study, an attempt was made to identify novel transcription factors by pulling down FLAG-tagged RNAP in M. smegmatis. In the experimental design for pull down assay, two sets of cell lysates were prepared –one set was treated with benzonase to remove any nucleic acids bound to RNAP while the other set was untreated. Several proteins were selected for further analysis and four previously uncharacterized proteins were also able to reciprocally pull down RNAP when used as bait. These proteins are now being extensively characterized. The results will be presented, extending our knowledge of the bacterial transcription machinery.

Characterization of MoaB2 from Mycobacterium smegmatis: a novel binding factor of mycobacterial SigmaA

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Mycobacterium is a genus of medicinally important bacteria with a number of human pathogens. Understanding the mycobacterial gene expression is necessary to develop new antibacterial approaches to fight diseases such as tuberculosis. The central enzyme of bacterial gene expression is RNA polymerase whose core is catalytically active but to initiate transcription it needs a sigma factor. SigA is the primary sigma factor responsible for transcription of housekeeping genes but it is not known how exactly SigA levels are regulated in mycobacteria. In Mycobacterium smegmatis, we identified a new protein associated with SigA, MoaB2. It is predicted to be involved in the biosynthesis of molybdopterin, an essential cofactor of a diverse group of redox enzymes. By several approaches, we demonstrate that MoaB2 binds to SigA but not to any other sigma factors in the mycobacteria. Consistent with the existence of SigA-MoaB2 complex, we see a moderate decrease in SigA dependent transcription in the presence of MoaB2 in vitro. In vivo, however, MoaB2 does not seem to play a significant inhibitory role, as its overexpression does not affect growth. Rather, MoaB2 seems to protect SigA as it increases its biological half-life. Finally, we solved the 3D structure of MoaB2 by crystallography to provide a basis for further studies. Taken together, MoaB2 represents a new binding partner of SigA in mycobacteria and extends thus our knowledge of the architecture of their transcriptional machinery.

Effects of reactivation of RNA interference pathway in mammals

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RNA interference (RNAi) is defined as sequence-specific mRNA degradation mediated by long double stranded RNAs (dsRNAs). The RNAi pathway is found in many eukaryotes and is initiated by the endoribonuclease Dicer, which cleaves long double-stranded RNA molecules into ~22 nucleotides-long short interfering RNAs (siRNAs). RNAi is an ancestral antiviral innate immunity and genome defense system in plants and invertebrates, whereas canonical RNAi and physiological function in mammals appear restricted to some rodent oocytes, which express a Dicer isoform lacking the N-terminal helicase domain, which is adapted to siRNAs production. This truncated isoform, denoted Dicer O , appears to be the main factor responsible for highly active endogenous RNAi activity observed in mouse oocytes. In order to assess whether RNA interference could be restored in soma, we generated a genetically modified mouse model where the full-length Dicer was substituted with Dicer O equivalent lacking the helicase domain (ΔHEL1). While homozygous Dicer ΔHEL1 mice die perinataly, heterozygotes are viable and fertile and Dicer ΔHEL1 protein variant is stably expressed in mouse tissues. Here we report phenotype analysis of Dicer ΔHEL1 mice with focus on their ability to process long dsRNAs into siRNAs and activate RNAi in vivo.

BRAT1 links Integrator and defective RNA processing with neurodegeneration

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Mutations in BRAT1 have been associated with neurological disorders characterized by phenotypes with varying levels of clinical severity. However, the underlying molecular mechanisms of disease pathology remain poorly understood. Recently, we identified a novel homozygous missense c.185T>A variant in BRAT1 characterized by a much milder phenotype than previously reported, which greatly reduced the level of the BRAT1 protein in patient-derived cell lines. Surprisingly, our data show that this decreased level of BRAT1 does not interfere with ATM kinase activation or DNA damage response, suggesting that BRAT1 has another functional role, which if affected similarly leads to neurological disease.
Excitingly, we identify BRAT1 as a novel binding partner of the catalytic RNA endonuclease heterodimer INTS9/INTS11 of Integrator complex. We show that insufficient and/or incorrect interaction of the BRAT1 protein with the INTS9/INST11 heterodimer impedes a processing of Integrator target RNAs. Importantly, Integrator dysfunction is also evident in cells obtained from BRAT1-related neurological disease patients. Taken together, our results support the model in which BRAT1 interacts with and stabilizes the INTS11, thereby maintaining the structural and functional integrity of the Integrator core cleavage heterodimer. Collectively, we suggest that defects in BRAT1 interfere with proper Integrator functions, leading to aberrant RNAs and proteins expression, resulting in neurodegeneration.

Retinitis pigmentosa-linked mutations alter the RNA binding of PRPF8 and SNRNP200

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Retinitis pigmentosa (RP) is a hereditary disorder caused by mutations in a hundred different genes including those that encode proteins important for pre-mRNA splicing. Most RP-associated mutations in splicing factors reduce either their expression, stability or incorporation into functional splicing complexes. Here, we studied four RP mutations in two splicing factors - PRPF8 (F2314L and Y2334N) and SNRNP200 (S1087L and R1090L) - that neither destabilize nor reduce the incorporation of the mutated proteins into splicing complexes and it is unclear how these mutations affect protein functions. Using iCLIP, we found that both mutations in the RNA helicase SNRNP200 change its interaction with U4 and U6 snRNAs. Mutated SNRNP200 has a significantly broader binding profile within the U4 region upstream of the U4/U6 stem I, which strongly suggests impaired snRNA unwinding activity. iCLIP also revealed reduced binding of mutated PRPF8 to pre-mRNA. This finding was further confirmed by FRAP that showed lower interaction of RP variants of PRPF8 with RNA. Further analyses of the Y2334N variant of PRPF8 in retinal pigment epithelial cells revealed a mis-splicing of selected introns and altered expression of hundreds of genes, suggesting that changes in the expression and splicing of selected genes are the main driver of retina degeneration in RP.

Complementation analysis of ribosomal and extraribosomal functions of Rpl22 in Saccharomyces cerevisiae

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RPL22A and RPL22B are paralogous ribosomal protein genes which were shown in Saccharomyces cerevisiae to be intergenicaly and asymmetrically regulated. Rpl22 proteins bind to the introns of their genes and differentially inhibit splicing. This is how the cells maintain a ratio of A and B mRNA and protein isoforms. Rpl22 protein is positioned peripherally on the large subunit, close to the protein exit tunnel, contacting loops 57 and 59 of 25S rRNA. The ribosomal function of Rpl22 is not yet understood; the deletion of both paralogs confers only a moderate growth phenotype.

We tested both functions of Rpl22A/B in S. cerevisiae using a panel of fungal and human Rpl22 proteins. Proteins from Candida albicans, Candida glabrata, Debaryomyces hansenii, Kluyveromyces lactis, Schizosaccharomyces pombe, Yarrowia lipolytica, and H. sapiens were expressed in WT and rpl22aΔ rpl22bΔ S. cerevisiae strains and their capacity to complement the growth phenotype of rpl22ΔΔ strain was measured. We then analyzed the RNA binding propensities of these proteins using a yeast 3-hybrid system with either 25S rRNA fragment spanning the Rpl22 binding region or the RPL22B intron fragment. The capacity to inhibit splicing in vivo was assessed using a reporter plasmid carrying RPL22B intron. Our data suggest that the ribosomal function tolerates fewer amino acid alterations than the evolutionarily novel extra ribosomal capacity to block pre-mRNA splicing.

New insights into the mechanics of translational control of human ATF4 – the study of the uORF2 DRACH methylation motifs.

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ATF4 is a regulatory protein functioning as essential transcription factor during integrated stress response. Its translation is induced via an alternative translation reinitiation (REI) mechanism in response to eIF2α phosphorylation, and stress-induced ATF4 acts as a master regulator of stress-responsive genes to ensure stress adaptation and cell recovery. Recently, we and others showed that in addition to the two well-known players of this so-called delayed REI process – the short uORFs 1 and 2 - also the further upstream uORF0 has a regulatory role in human ATF4 translational control. Moreover, REI of mouse ATF4 was shown to be subjected to regulation by mRNA methylation in the form of N6-methyladenosine (m6A), which controls ribosome scanning and subsequent start codon selection. Here, we examined human ATF4 transcript leader and the uORF2-ATF4 overlapping region for the presence of potential m6A methylation motifs DRACH and found A225, A235, A286 and A326 as possible targets for m6A methylation. Using T3-ligation-qPCR, we have examined these A residues for quantitative changes in their m6A status under stress vs. non-stress conditions. Simultaneously, using quantitative Western blotting of HA-tagged ATF4 protein expressed transiently from pDNA harboring specific mutations of these As, we have compared ATF4 protein levels under the same conditions. We will present findings addressing what role the m6A methylation plays in the overall translational control of human ATF4.

5’ poly(A) sequences affect the rate of translation initiation of yeast mRNAs

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Translation of eukaryotic mRNAs into the primary protein structure is a highly important part of gene expression. Translation initiation now is well-defined process without which translation itself could not take place. Translation initiation is not only ensured by a number of mRNA modifications and initiation factors, but the structure of eukaryotic transcripts itself also plays an important role. Transcripts with a highly specific sequence have been described in the yeast *Kluyveromyces lactis*. These mRNAs, expressed from the linear cytoplasmic plasmids pGKL1 and pGKL2, contain neither the cap moiety at their 5’ end nor the 3’ poly(A) tail. In contrast, these transcripts contain up to 18 non-template adenosine residues at their 5’ ends.
Here we show the effect of these non-template adenosines on translation rates of plasmid-specific mRNAs. We have prepared a unique reporter system based on the precise modification of pGKL plasmids by inserting a reporter cassette into these plasmids under the control of plasmid-specific pGKL promoters that give rise to distinct variants of the 5' ends of the mRNA. One reporter cassette gives rise to transcript carrying the cap structure at the 5´-end of the reporter mRNAs, mRNAs transcribed from the second reporter cassette contain non-capped mRNA with shorter sequences of non-template adenosine residues at the 5´end, and finally uncapped sequences with long non-template 5´polyA leaders are transcriber from the third reporter. Results of reporter activity assay indicate an effect of non-template 5´polyA sequences on translation rate compared to control (capped) reporter mRNA.

MORG1 may regulate assembly of stress granules through bidirectional regulation with its natural antisense transcript DHPS

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Mitogen-activated protein kinase organizer 1 (MAPK organizer 1; MORG1) is a WD40 protein that was first identified as a scaffold of the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway. MORG1 is also known for its role in the regulation of cellular response to hypoxia via its interaction with prolyl hydroxylase 3 (PHD3). An involvement of MORG1 in pre-mRNA splicing has been proposed as well. Although MORG1 is an essential protein in eukaryotes, its cellular functions are not very well explored.

We recently observed MORG1 localizing into stress granules (SGs) under arsenite-induced oxidative stress in epithelial MDCK cells. That suggested possible involvement of MORG1 in translation; considering also the fact, that MORG1 has a natural antisense transcript encoding for deoxyhypusine synthase (DHPS). An overlap at their 3´UTRs allows the MORG1 and DHPS mRNAs to form duplexes, resulting in an increased stability of both transcripts. DHPS is an enzyme catalyzing first step of hypusination, an unusual posttranslational modification that occurs only in a single cellular protein, eukaryotic translation initiation factor 5A (eIF5A). Hypusination is a key modification for eIF5A to promote not only elongation, but also polysome disassembly and SGs formation after arsenite-induced stress conditions. We suggest that via its antisense regulation of DHPS mRNA stability leading to eIF5A hypusination, MORG1 may contribute to SGs assembly.

Role of yeast MCTS1/Tma20, DENR/Tma22 and eIF2D/Tma64 in ribosome recycling

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Translation is divided into distinct phases: initiation, elongation, termination and ribosome recycling, and many of the mechanisms involved – especially in ribosome recycling – still elude us. During ribosome recycling, splitting of the ribosomal subunits is mediated by ABCE1/yeast Rli1, whereas ejection of tRNA from the small subunit has been shown to be mediated by i) canonical translation initiation factors, or ii) in vitro by eIF2D and the homologous DENR/MCTS1 complex. Yeast homologues of these three proteins – Tma64/eIF2D, Tma20/MCTS1 and Tma22/DENR serve as general 40S recycling factors, with 40S recycling activity mediated mainly by the Tma20/Tma22 complex. It has been shown that the identity of the ORF's penultimate codon modulates the dependency of each recycling event on these factors, suggesting possible importance of Tma20/-22 interaction with deacylated tRNA. Using an established reporter system, we explore the relation of penultimate codon identity with 40S recycling and the role of each factor. Consistently with recent literature, we find that deletion of these factors causes a 40S recycling defect. Moreover, certain penultimate codons enhance or lessen this recycling defect, thus confer a different degree of Tma-dependence. Additionally, these factors seem to cooperate in a manner consistent with the assertion that Tma20/-22 bear the brunt of 40S recycling activity, with Tma64 performing a yet unknown role.

Novel RNA molecules interacting with the bacterial transcription machinery

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Regulatory 6S RNA molecules that interact with the RNA polymerase are widespread among bacteria. We discovered a new type of regulatory RNA in mycobacteria, named it Ms1 and showed that Ms1 regulates the amount of RNA polymerase in nonpathogenic Mycobacterium smegmatis. In addition, we found Ms1 homologs among other actinobacteria using bioinformatic search. This phylum includes severe human pathogens (for example Mycobacterium tuberculosis, Mycobacterium leprae, Corynebacterium diphtheria), industrially important producers of amino acids (Corynebacterium glutamicum) and antibiotics (Streptomycetes) or probiotic bacteria (Bifidobacterium). We performed RIP-seq (RNA immunoprecipitation coupled with next-generation sequencing) and identified a complete set of regulatory RNAs interacting with the transcription machinery in several bacterial species - Mycobacterium smegmatis, Streptomycetes celicolor and well established model organism Bacillus subtilis. Our data show that in addition to 6S and Ms1 RNA, other RNAs associate with the different forms of bacterial RNA polymerase. These novel RNAs expand the portfolio of possible mechanisms of bacterial transcription regulation. We propose that 6S RNA and Ms1 were the first RNAs to be identified due to their high abundance; however, other; less abundant regulatory RNAs are waiting to be discovered.

The effect of subunit composition of RNA polymerase on sporulation in B. subtilis

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Certain bacterial species can survive in hostile and even extremely adverse conditions due to their ability to sporulate. This process depends on precisely temporally and spatially regulated gene expression, which is mediated by RNA polymerase (RNAP), the central enzyme of transcription, and a cascade of alternative σ factors. Here we describe the effects of small, non-essential subunits of RNAP, δ and ω, on sporulation in the model Gram-positive bacterium Bacillus subtilis. Experiments revealed a synergistic interplay between these subunits, the RNAP core, σ factors, and gene expression and provided insights into their role in sporulation. A model of their involvement in the sporulation cascade will be presented and discussed.

Effect of ADAR1 enzyme on hepatitis C virus life cycle

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Hepatitis C virus (HCV) is a virus of the family Flaviviridae whose genome consists of ,,+RNA" molecule. It causes the disease hepatitis C, which affects tens of millions of people worldwide. Several ambigious works focused on the relationship between HCV and the enzyme adenosine deaminase acting on double-stranded RNA 1 (ADAR1) were published in the past. This dimeric double-stranded RNA binding enzyme is a part of innate immunity and causes catalytic conversion of adenosine to inosine, which is recognized by cellular mechanisms as guanine, which leads to mutations in the affected dsRNA molecule. The works published so far attribute an antiviral function to the ADAR1 enzyme in the context of HCV infection. However, vectors containing the entire HCV genome were not used in these works, and a cell line with deletion of the ADAR1 gene has never been used so far. To gain a deeper understanding of the relationship between the HCV virus and the ADAR1 enzyme, HCV permissive Huh7.5 cell lines with ADAR1 gene deletion were prepared. By in vitro transcription of plasmid pJFH1-pUC, containing the complete HCV genome and subsequent transfection of viral RNA into Huh7.5 cell line, infectious virus particles of HCV virus were successfully prepared and their titer was subsequently determined. These viral particles were able to infect both Huh7.5 wt and Huh7.5 ADAR1KO cell lines, but the effect of ADAR1 enzyme on HCV life cycle is still yet to be determined.

HelD regulates RNA polymerase level in Mycobacterium smegmatis

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HelD binds RNA polymerase (RNAP) in Bacillus subtilis and Mycobacterium smegmatis, where it dissociates stalled transcription complexes. We show that overexpression of HelD in M. smegmatis decreases the total amount of RNAP protein and the level of Ms1 RNA (RNAP-associated small RNA) in stationary phase of growth. The two main subunits of RNAP, beta and beta´, are encoded by rpoB-rpoC genes which are located in one operon. Using chromatin immunoprecipitation coupled with next generation sequencing (ChIP-seq), we found that HelD protein is highly enriched at the rpoB-rpoC promoter. rpoB-rpoC mRNA has an unusually long and structured 5´UTR of unknown function that could be a target of HelD regulation.
In addition, we identified a complete set of genes that are occupied by HelD protein by ChIP-seq. HelD associates mainly with the genes encoding structured RNAs – rRNAs and tRNAs. HelD might help to RNAP to transcribe genes encoding highly structured RNAs, or, help to release stalled RNAP from these genes. We generated ΔhelD strain to compare the RNAP profiles with the wild type strain by ChIP-seq. We assume that the genome-wide occupancy of RNAP will differ in ΔhelD strain compared to the wild type.

REVERSE TRANSCRIPTASE FINGERPRINT METHOD AS A SELECTIVE PROFILING TECHNIQUE FOR THE IDENTIFICATION OF NPnN-RNAs

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Recently, a new class of 5' RNA caps, with structure of dinucleoside polyphosphates (NpnNs), was discovered in E. coli. Free NpnNs were discovered 50 years ago in all types of cells. It is known that their concentrations increase under the stress. Nevertheless, the intracellular role of NpnNs is still enigmatic. Preliminary data from our laboratory show that NpnN-RNAs are also present in mammalian tissue cell culture. However, nothing is known about the types of RNA bearing NpnN caps and how they influence cellular reactions to stress. Therefore, it is essential to develop a selective profiling technique that allows for identification of RNA types capped with NpnNs. It is known that reverse transcription of internal modifications of RNA leads to synthesis of cDNA containing information on the modification in the form of misincorporation, arrest, or nucleotide skipping events, representing an RT-signature (fingerprint). Because nothing is known about the reading and recognition of these NpnN caps by RTs, we want to exploit this RT fingerprint method for the preparation of RNA-seq libraries. Model RNA bearing different 5` RNA caps (NAD, Ap_2-$_6$A, Gp_2-$_4$G) will be used as substrates for a combination of RTs. Then, we will prepare RNA-seq library suitable for Oxford Nanopore Technology. If we observe significantly different behavior of various RT with our NpnN RNA caps by bioinformatic analysis, the method will be applied on RNA from real samples (E. coli or human tissue cells).

Roles of ω and δ subunits from Bacillus subtilis RNA polymerase in transcription

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RNA polymerase (RNAP) catalyzes transcription of DNA into RNA. RNAP consists of a core that contains two α subunits, one of each β,β‘ and ω. These subunits are conserved in all bacteria. Gram-positive Firmicutes contain two additional subunits, δ and ε. The ω subunit (molecular weight 7.64 kDa) is the smallest subunit of the core RNAP where it binds to the β‘ subunit. In Gram-negative bacteria, the ω subunit is important for promoter selection and for folding and integrity of RNAP. However, the exact role of the ω subunit in Gram-positive bacteria remains unknown. Here, I present the first view of the effects of the ω subunit from Bacillus subtilis (Firmicutes) on transcription. First, I describe an optimized protocol for ω purification. Next, I show the effects of ω and δ on in vitro transcription with the primary σA factor and alternative σF and σE factors. These alternative σ factors regulate sporulation in B. subtilis. The experiments have revealed that ω and δ synergistically stimulate transcription; the effect is most pronounced for RNAP•σF. This is fully consistent with the detrimental effect of the absence of ω and δ on sporulation (see poster by Kálalová et al.). Finally, future experiments will be outlined.

Ribosomal A-site interactions of tRNA-Gln driving stop codon readthrough

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Translation is a fundamental process for the cell, which is continuous and without which there would be no life. One of the key components in the translation process is the tRNA molecule which delivering amino acid residues to the ribosome during initiation and elongation phases, it also plays a crucial role in translational control processes at various levels, such as the programmed stop codon readthrough. During the programmed stop codon readthrough, there is a competition between the release factor and a near-cognate tRNA. This tRNA is able to incorporate into one of the three stop codons, leading to repression of termination and allowing translation to continue until the next in-frame stop codon. Here we show with the specific iso-acceptor of yeast tRNAGln , that not only the level of the coplementarity between appropriate tRNA's anticodon and stop codon, but also the nature of the bases forming the anticodon stem of the tRNA and their contact with ribosomal proteins located in the A-site critically affect the ability of the tRNA in the stop codon readthrough.

m7Gp4Gm-RNA: Development of Selective CaptureSeq Method

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Bacterial adaptation to stress is essential for their survival and RNA turnover control plays a pivotal role in the process. Increasing evidence suggest that RNA 5'-end modifications represent additional tuning level of their metabolism. We have recently reported that noncanonical m7Gp4Gm cap, substantially increases on E. coli sRNA in response to stress conditions (1). m7Gp4Gm-RNA showed resistance to RNA 5'-pyrophosphohydrolase, enzyme decapping non-methylated RNA caps, indicating an important role of m7Gp4Gm cap in RNA faith. To understand the purpose of m7Gp4Gm cap in broader biological context, new techniques to identify m7Gp4Gm-capped RNA sequences are needed.
Here, we present newly developed immunoprecipitation-based method that was applied on sRNA isolated from E. coli harvested at exponential and late stationary phase of growth. Captured RNA was reverse transcribed into cDNA and sequenced on Illumina. sRNA treated with a decapping enzyme prior immunoprecipitation was used as negative control.
Bioinformatic analysis of sequencing data from six biological replicates revealed several RNAs significantly enriched compared to controls. In order to validate the method, we are currently pulling-down selected RNAs to confirm presence of m7Gp4Gm as the RNA cap by LC-MS. We believe that revealing m7Gp4Gm-capped RNA sequences will help us to understand how E. coli respond to stress by selective RNA capping.
1. Hudeček, O.; Benoni, R.; et al. Nat Commun 2020, 11, 1052

Importance of N6-methyladenosine levels for cellular homeostasis and DNA repair

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N6-methyladenosine (m6A) and N6,2'-O-dimethyladenosine (m6Am) are modifications present across various classes of RNA in all higher eukaryotes. m6A(m) affect nearly every phase of the RNA life cycle, from nuclear processing to cytoplasmic RNA surveillance. We have recently characterized in cellulo protein interactomes of the key enzymes engaged in the m6A and m6Am pathway, using proximity labelling and mass spectrometry. These studies pointed to physical interactions between the m6A and DNA replication/repair factors.
Here, we present follow-up studies addressing the relevance of the identified interactions in a genome, proteome, and RNA-interactome-wide context. While our findings widely confirm our data on the interactome of the m6A eraser ALKBH5, they also highlight new, unprecedented functions for the eraser protein FTO. We report here new isoform-specific functions of FTO in human cells, pointing towards its engagement at the intersection of cellular homeostasis and DNA repair.

he expression and enzymatic activity of human ISG20L2 exoribonuclease

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Posttranscriptional modifications and RNA stability play important role in the regulation of gene expression and the overall cellular metabolism. Modifications can ensure the right transport to cytoplasm and translation of coding RNAs but at the same time, 3' end processing of non-coding RNAs play a vital role in controlling their fate in a cell. The addition of non-templated 3'-terminal nucleotides (tailing) acts in both; RNA maturation and RNA decay. RNA tailing is carried out by the so called terminal nucleotidyl transferases (TENT protein family) with diverse NTP specificities. These tails can be then recognised by specialized exoribonucleases such as the exosome, DIS3L2, Usb1 or hEXO1. Here we aim to tackle the mechanisms of another mammalian exoribonuclease ISG20L2 that has been linked to rRNA processing in the nucleolus (Coute et al., 2008). We aim to describe its enzymatic and RNA binding properties in vitro. Preliminary results of the protein expression and purification and in vitro degradation essays will be presented.

Ribosome profiling and Sel-TCP-Seq as tools for understanding the role of individual eIF3 subunits

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Being composed of 12 subunits the eucaryotic translation initiation factor 3 is the largest and most complex of translation initiation factors in humans. Our lab has previously shown that downregulation of individual eIF3 subunits leads to disruption of the 12-subunit eIF3 complex and formation of distinct eIF3 subcomplexes. Knocking down individual eIF3 subunits also affects cell proliferation and overall translation efficiency to a various level. Since dysregulation of many eIF3 subunits has been reported in different cancer types, we are interested in monitoring changes in dynamics of translation initiation complexes, in mRNA expression pattern and in their translation efficiency when individual eIF3 subunit level is manipulated. Towards this end we are using ribosome profiling and selective translation complex profile sequencing (Sel-TCP-Seq). In ribosome profiling elongating ribosomes are stalled by cycloheximide treatment and mRNA footprints of these ribosomes are isolated and sequenced. Sel-TCP-Seq builds upon ribosome profiling but in addition to elongating ribosomes footprints of scanning translation initiation complexes are also sequenced. Subjecting cells in which individual eIF3 subunits were either downregulated or overexpressed to ribosome profiling or Sel-TCP-Seq will allow us to investigate the role of individual eIF3 subunits and eIF3 subcomplexes.

Argonaute 2 adaptations in mouse oocytes

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Argonaute proteins are key factors in effector complexes dedicated to RNA silencing. Mammals employ four AGO proteins (AGO1 – AGO4), which primarily function in the microRNA (miRNA) pathway. Remarkably, AGO2 retains endonucleolytic activity and can support RNA interference (RNAi). In mammals, endogenous RNAi is an essential pathway for mouse oocytes because of an oocyte-specific short Dicer isoform, DicerO, which has higher processivity of double-strand RNA (dsRNA). RNA sequencing revealed specific AGO2 adaptations in mouse oocytes: 1) emergence of an alternative exon-1, producing an isoform (AGO2ooc) with different amino acid composition in the N-terminus; and 2) a novel 3' terminal exon in intron-3 generated by a mouse-specific MT element. Here, we aim to assess whether these AGO2 adaptations are relevant for mouse oocyte biology. Notably, NIH3T3 cells expressing the oocyte-specific N-terminus (Ago2ooc/ooc) showed reduced RNAi-like (2-5 fold) but indistinguishable miRNA-like activity when compared to WT cells in reporter assays. Currently, we are generating mouse models whose oocytes would express the somatic exon-1 instead of the oocyte-specific exon-1, or N-terminal variants with mutations in putative key amino acid residues. Finally, we are producing mice with insertion of full-length Ago2 cDNA to circumvent the expression of the truncated AGO2 isoform and boost the expression of full-length AGO2 in mouse oocytes.

NON-CANONICAL RNA CAPS AFFECT THE RNA TURNOVER UNDER STRESS CONDITIONS IN E. COLI

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The discovery of the new class of 5´ RNA caps in bacteria opened new ways for the study of the RNA and its functions.Our study shows that NpnNs,also called alarmones,are incorporated into RNA by RNApol during transcription depending on the metabolic status of the cell.Even though, we observed in vitro incorporation of free NpnNs into RNA by RNApol,we cannot exclude the possibility that these caps are formed on RNA by some unknown capping enzyme.Therefore,the first task is to find correlation between free forms of NpnNs and NpnN-RNA in cell.We develop LC-MS technique for identification of free NpnNs in any type of cell.In addition,by LC-MS analysis of isolated RNA,we also detected methylated forms of these RNA caps increasing under stress conditions.We found that not all decapping enzymes are able to cleave the detected methylated forms of RNA caps suggesting further regulatory mechanisms,e.g.modulating RNA turnover.So, we focus on searching for methyltransferases responsible for methylation of cellular NpnN-RNA.For this purpose,we identified different tRNA methyltransferases that might play a role also in the methylation of RNA caps.Thus,we isolated RNA from the methyltransferase knockout E.coli strains and we analysed it using LC-MS.Consequently,we perform in vitro experiments to study the methylation activity.Overall,this work should help us to understand the formation and the role of NpnN RNA caps in the stability of RNA and the cellular reaction to stress.

Analysis of translation efficiency through polysome profiling in different phases of cell cycle

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Cell cycle is one of the highly regulated processes in an organism due to its critical role in growth and development. Protein levels at each of these phases are tightly regulated at different levels to ensure continuous cell cycle progression. Regulations occurring due to alternative transcription, splicing and polyadenylation lead to the production of multiple RNA transcript isoforms. These in turn, have different translation efficiency due to diverse features of their untranslated regions (UTRs). The role of transcript isoforms in regulating cell cycle has been unappreciated, compared to the genome wide studies on cell cycle. We have optimized a method to study the transcript isoforms of different phases of cell cycle combining flow cytometry and polysome profiling. We show that by fixing the polysomes by protein-protein and protein-RNA crosslinking, we are able to sort the different phases of cell cycle, which can be distinguished by DNA staining. We have further applied Scarce Sample Polysome Profiling (SSP-profiling), a technique previously developed by us, which allows downstream RNA-seq to identify polysome associated transcripts with scarce sample. This enables quick sorting, thus eliminating the possibility of polysome dissociation due to sorting induced delay in processing. Our study will help understanding the role of transcripts in cell cycle regulation with minimum to no influence due to external factors.

ChIP seq analysis for the leading mycobacterial transcription factors: CarD , RbpA and a novel transcriptional regulator, CrsL.

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The mycobacterial transcriptional machinery comprises two global transcription regulators binds to RNAP- CarD, RbpA. Both CarD and RbpA stabilize the open complex during transcription and are important for the sensitivity of RNAP to rifampicin; the first-line drug against tuberculosis. We discovered a new transcription factor that binds to CarD-RNAP complex and named it CrsL. Our data shows that CrsL regulates CarD level in the stationary phase and reveals a new mechanism of transcriptional regulation in mycobacteria.
We performed Chromatin Immunoprecipitation followed by next-generation sequencing (ChIP-seq) for CarD and RbpA transcriptional regulators in Mycobacterium smegmatis. In addition, we did this experiment for CrsL to see if it associates with the same promoters as CarD/RbpA.
Furthermore, we developed a webpage for visualizing our ChIP-seq data in addition to other sequencing data (RNA-seq) which we previously published. This webpage will be useful to visualize the expression profile of different genes compared to the presence of CarD/RbpA at these genes within M. smegmatis genome. Therefore, it will provide novel insights into the control of mycobacterial transcriptional regulation in order to develop new specific anti-tuberculosis drugs.

Mass spectrometry – based identification of 5'-RNA caps

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It is well known that most of the RNAs are post-transcriptionally modified on the nucleobases or ribose. Increased sensitivity of next-generation sequencing and especially mass-spectrometry based analysis revealed new class of modifications on 5' end of RNA, which are called RNA caps [1]. They are mostly derived from metabolites, cofactors and dinucleotide polyphosphates [2]. It is believed that their presence can affect the RNA stability, cellular metabolism and even mRNA translation, however their exact role is still not well described. The physicochemical properties of these molecules (high hydrophilicity, acidic phosphate functional groups, nucleobases) makes their analysis very challenging. This work will present workflow for qualitative and quantitative analysis of (non)canonical 5'-RNA caps in bacteria and mammalian tissue cell cultures. In all digested RNAs we have observed signals of different canonical and non-canonical caps such as NAD, CoA, dinucleotide polyphosphates and their methylated analogues. Their structure was validated based on the precise retention time, m/z ratio and compared to the commercial standards. In other cases, fragmentation spectra were acquired to confirm the identity of the cap.

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2. Hudeček, O., Benoni, R., Reyes-Gutierrez, P.E., Culka, M., Šanderová, H., Hubálek, M., Rulíšek, L., Cvačka, J., Krásný, L. and Cahová, H. (2020), Nature Comm., 11, 1052.

Dissolution of Heat shock Stress Granules in S. cerevisiae: The faster the better!

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After robust heat shock, exponentially growing yeast cells form cytoplasmic foci called stress granules (SGs) that contain housekeeping mRNAs, proteins involved in translation, RNA metabolism and signaling. While composition and formation of the SGs induced by heat stress or other stress are largely known, their function in stress response is still poorly understood.
Here we show that modulating dissolution of SGs in time affects a cell recovery after stress release. Faster dissolution of SGs correlates with the faster cell recovery and conversely delayed dissolution of SGs correlates with the delayed cell recovery. Furthermore, the dissolution of heat shock-specific SGs is independent of the non-selective autophagy or granulophagy indicating that components of SGs are not degraded during recovery.
Altogether, our results support the hypothesis that stress granules serve as storage sites of functional messenger ribonucleoprotein particles whose effective dissolution after stress release supports cell recovery and stress survival.

Identification of m6A modification in viroid RNA using LC-MS and Nanopore direct RNA sequencing.

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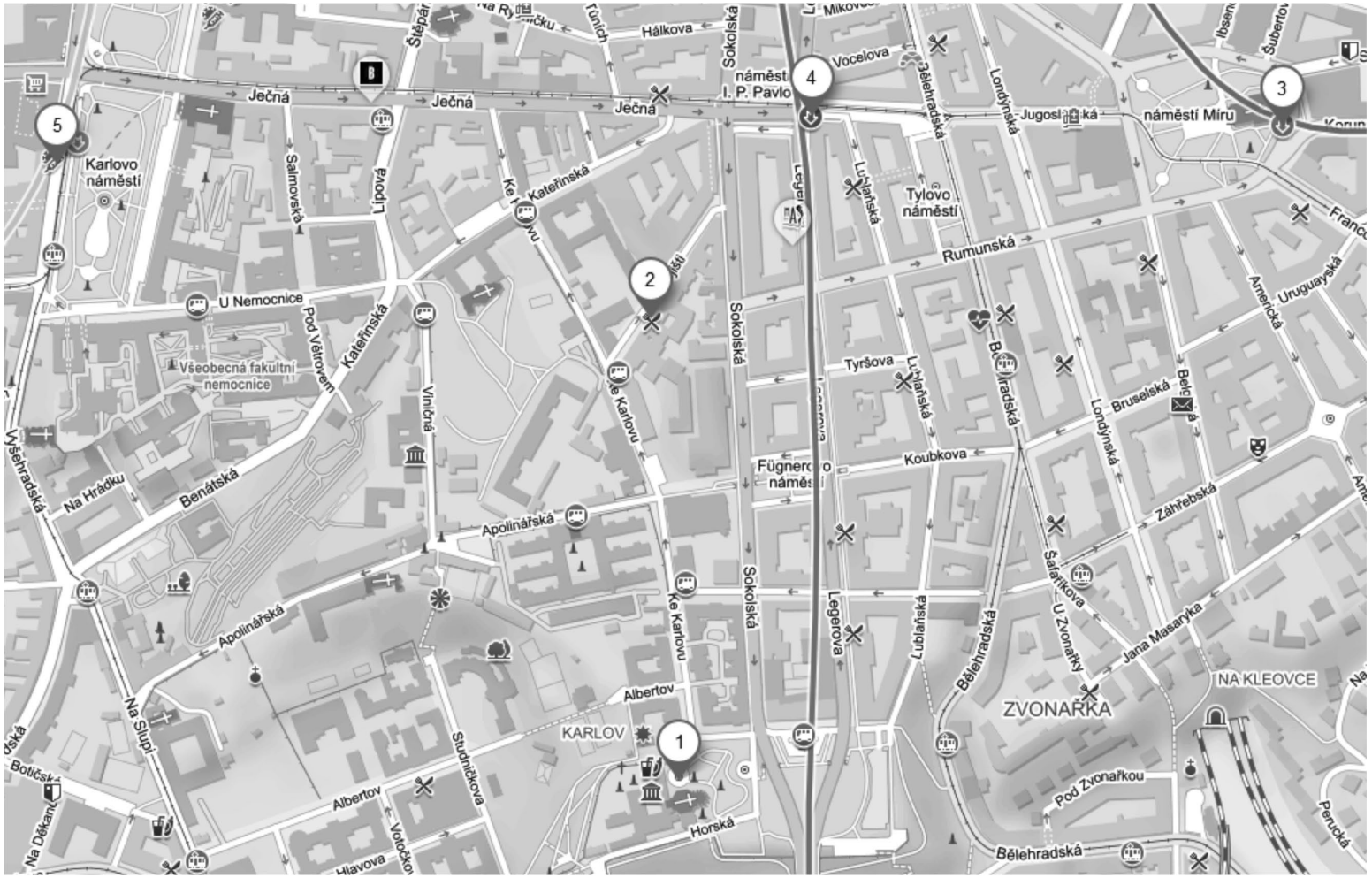
Viroids are small circular non-coding RNAs that act as infectious pathogens in higher plants. Despite viroid RNAs do not code for any proteins, viroids can autonomously replicate in plant cells. The replication can occur either in the nucleus (family Pospiviroidae), or in the chloroplasts (family Avsunviroidae). The structural features of viroid RNA determining the subcellular sorting into these two organelles are not well understood, however, RNA modifications were suggested to play a role. Using LC-MS analysis we detected m6A and sugar methylated nucleosides in viroid RNA. While we were not able to detect m6A modification in the ASBV RNA, the CEVd (Pospiviroidae) RNA contained one m6A modification per molecule of RNA. To precisely allocate the m6A modification into the structural context of the CEVd viroid RNA, we have implemented a protocol for Nanopore direct RNA sequencing of viroid RNA. Currently, we are analyzing the direct RNA-seq datasets and optimizing the base-calling pipeline. Once the position of m6A modification will be identified, functional studies will be performed to confirm its role in CEVd viroid biology.



1 - Czech Police Museum; Ke Karlovu 453/1, 120 00 Praha
2 - U kalicha; Na Bojišti 12-14, 120 00 Praha 2
3 - Náměstí Míru (metro Line A)
4 - I.P.Pavlova (metro line C)
5 - Karlovo náměstí (metro line B)