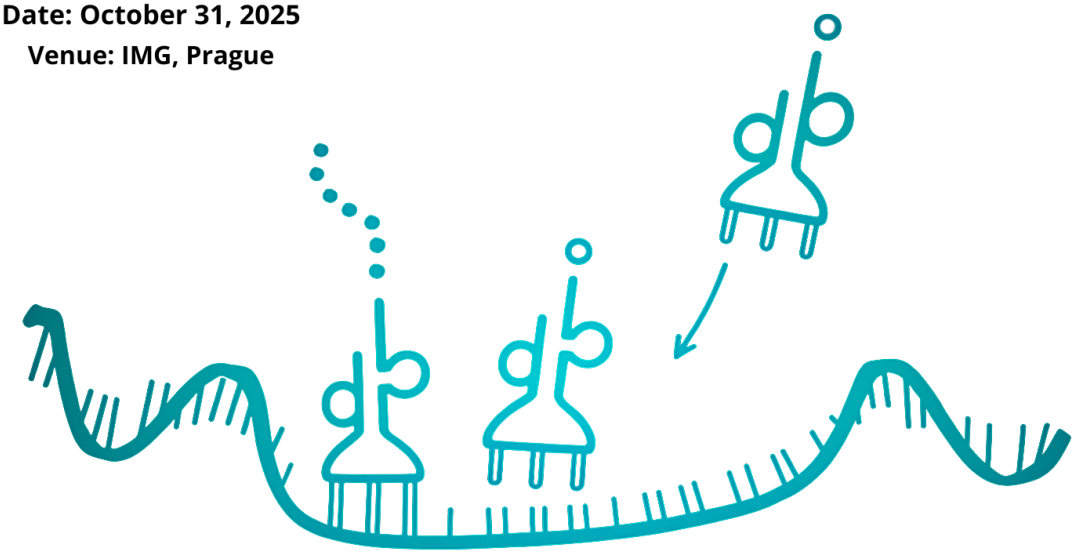
**Date: October 31, 2025**
**Venue: IMG, Prague**



# RNA CLUB

**Co-organizers: IMIC & IMG**

# ABSTRACT BOOK

# Oral presentations

## Enzymatic Synthesis of Modified RNA Containing 5-Methyl- or 5-Ethylpyrimidines or Substituted 7-Deazapurines and Influence of the Modifications on Stability, Translation and CRISPR-Cas9 Cleavage

**Tania Sanchez-Quirante**[1], **Erika Kužmová**[1], **Miguel Riopedre-Fernandez**[1], **Sebastian Golojuch**[2], **Pavel Vopálenský**[1], **Michal Hocek**[1]

*1. Institute of Organic Chemistry and Biochemistry Czech Academy of Sciences. Flemingovo nam.2, 16610 Prague 6, Czech Republic*
*2. Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Oxford OX1 3TA, UK*

**E-mail of the presenting author:** tania.sanchez@uochb.cas.cz

While over 150 natural RNA modifications have been identified, their impact extends beyond endogenous RNAs, influencing the function of synthetic RNAs, including single-guide RNAs (sgRNAs) for CRISPR-Cas9 and messenger RNAs (mRNAs) used in protein expression. Our lab previously demonstrated the ability of T7 RNA polymerase to incorporate small modifications into RNA. In this study, we synthesized a library of ten modified nucleoside triphosphates, including purines with methyl or ethyl groups at the C7 position, pyrimidines with modifications at C5, as well as deazaadenosine and deazaguanosine without additional substitutions. These modified nucleotides were incorporated into sgRNAs via *in vitro* transcription, and their impact on CRISPR-Cas9 cleavage efficiency and serum stability was evaluated. Additionally, modified mRNAs encoding Renilla luciferase were synthesized and assessed for translation efficiency *in vitro* using the Rabbit Reticulocyte System and in-cellulo following transfection into HeLa S3 cells. Stability was monitored by ddPCR, and luminescence was measured over time. These results help us understand how chemical modifications influence RNA functions with potential applications in gene editing and therapeutic mRNA design.

# Using structured libraries, selection and machine learning to rapidly explore the sequence space of a fluorescent deoxyribozyme

**Jaroslav Kurfürst[1,2], Martin Volek[1,3], Raman Samusevich[1], Tomáš Pluskal[1], Edward Curtis[1]**

*1. IOCB Prague*
*2. Department of Informatics and Chemistry, University of Chemistry and Technology*
*3. Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague*

**E-mail of the presenting author:** curtis@uochb.cas.cz

Finding ways to more comprehensively explore the sequence space of complex functional motifs is an important and unresolved question in nucleic acid engineering. Standard approaches use libraries in which a single variant of a motif is randomly mutagenized at a low level. This provides comprehensive coverage of sequence space over short mutational distances, but only limited information about more distant variants. Here we describe a new approach that uses libraries made up of sequences consistent with the constraints of a desired target motif. Functional variants are rapidly identified in a single round of selection followed by high-throughput sequencing, and rules relating sequence to function elucidated using machine learning. This method was tested using a fluorescent deoxyribozyme recently discovered in our group called Aurora. Single-step selections showed that a secondary structure library based on Aurora contained approximately 40-fold more unique catalytic sequences than one generated by random mutagenesis. Furthermore models developed by machine learning could quantitatively predict read numbers and identify the most active variants using small subsets of sequences as training sets. By combining secondary structure libraries, selection and machine learning in this way, sequence space can be explored far more quickly and efficiently than in standard approaches.

# The ω and δ subunits of RNA polymerase control sporulation in Bacillus subtilis

**Debora Pospíšilová[1], Klára Mikesková[1], Jiří Pospíšil[1], Anna Mária Palliesková[1], Radka Holbová[2], Jana Moravcová[2], Martin Převorovský[3], Markus C. Wahl[4,5], Marek Schwarz[6], Hana Šanderová[1], Ivan Barvík[7], Libor Krásný[1]**

*1. 1 Department of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sciences, Prague CZ-14220, Czech Republic*
*2. 2 CEITEC-Central European Institute of Technology, Masaryk University, Brno CZ-62500, Czech Republic*
*3. 3 Department of Cell Biology, Faculty of Science, Charles University, Prague CZ-12800, Czech Republic*
*4. 4 Laboratory of Structural Biochemistry, Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 6, Berlin D-14195, Germany*
*5. 5 Macromolecular Crystallography, Helmholtz-Zentrum Berlin für Materialien und Energie, Albert-Einstein-Str. 15, Berlin D-12489, Germany*
*6. 6 Department of Bioinformatics, Institute of Microbiology, Czech Academy of Sciences, Prague CZ-14220, Czech Republic*
*7. 7 Faculty of Mathematics and Physics, Institute of Physics, Charles University, Prague, Czech Republic.*

**E-mail of the presenting author:** deborakalalova@seznam.cz

Some bacterial species can survive in hostile and even extreme 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 explore the effects of small, non-essential subunits of RNAP, δ, ε, and ω, on sporulation in the model Gram-positive bacterium *Bacillus subtilis*. The combined absence of ω and δ reduces sporulation by 99 %, while single deletions of the respective genes have little to no effect. Transcriptomic data reveal downregulation of hundreds of mostly sporulation-related genes, including moderately decreased expression of Spo0A, the master regulator of entry into sporulation. Experiments *in vitro* show that the transcription machinery dependent on alternative σ factors (namely σH and sporulation-specific σF) is more negatively affected by the absence of ω and δ than transcription dependent on the vegetative σ factor, σA. This results in the absence of an asymmetric septum and leads to reduced sporulation. This study thus reveals a synergistic interplay between ω and δ, the RNAP core, σ factors, and gene expression and provides insights into the role of small subunits in sporulation.

# CrsL is a novel transcription regulator in actinobacteria

**Viola Vaňková Hausnerová[1], Mahmoud Shoman[1,2], Martin Černý[3], Jitka Jirát Matějčková[1], Marek Schwarz[2], Libor Krásný[2], Lukáš Žídek[3], Jarmila Hnilicová[1]**

*1. Faculty of Science, Charles University, Prague, Czech Republic*
*2. Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*
*3. Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic*

**E-mail of the presenting author:** viola.hausnerova@natur.cuni.cz

Transcription regulation is critical for adaptation and survival of bacteria. CarD is an essential transcription factor in mycobacteria involved in regulation of gene expression and a binding partner of mycobacterial RNA polymerase. We searched for CarD interaction partners in the model organism *Mycobacterium smegmatis* and identified a small protein which we named CrsL (MSMEG_5890). CrsL is a 5.7 kDa protein shown by NMR to be intrinsically disordered. Once bound to CarD, CrsL becomes partially structured. CrsL homologs are present in actinobacteria including pathogenic species such as *Mycobacterium tuberculosis*. CrsL interacts directly with CarD, also forms complexes togehther with RNAP, and controls CarD-RNAP association. ChIP-seq showed that CrsL associates with promoters of actively transcribed genes together with RNAP, sigma A, RbpA and CarD. CrsL is involved in the regulation of DesA desaturase (*MSMEG_5773*) and DEAD/DEAH-box RNA helicase *MSMEG_1930*, which are important for adaptation to cold stress. Furthermore, CrsL promotes the growth of *M. smegmatis* at elevated temperature and decreases growth at low temperatures. In summary, we have identified a novel transcription regulator in actinobacteria.

# Molecular insight into 5’ RNA capping with dinucleoside polyphosphates by bacterial RNA polymerase

**Valentina Serianni**[1], **Jana Škerlová**[1], **Tomáš Kouba**[1], **Hana Cahova**[1]

*1. Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 2, Prague 6, Czechia*

**E-mail of the presenting author:** valentina.serianni@uochb.cas.cz

RNA molecules capped with dinucleoside polyphosphates have been discovered in both bacterial and eukaryotic cells only recently. The likely mechanism of this specific capping involves the direct incorporation of dinucleoside polyphosphates by RNA polymerase, which serve as non-canonical initiating nucleotides. However, how these compounds bind into the active site of RNA polymerase during the initiation phase of transcription is unknown. We explored transcription initiation *in vitro*, using a series of DNA templates in combination with dinucleoside polyphosphates and model RNA polymerase from *Thermus thermophilus*. We observed that the transcription start site depends on the combination of the specific template and dinucleoside polyphosphate. Cryo-EM structures of transcription initiation complexes with dinucleoside polyphosphates revealed that both nucleobase moieties can pair with the DNA template. The first encoded nucleotide pairs in a canonical Watson–Crick manner whereas the second nucleobase moiety pairs non-canonically in a reverse Watson–Crick manner. Our work provides a structural explanation of the general mechanism by which dinucleoside polyphosphates initiate RNA transcription.

# FTO m6A RNA demethylase is important for proper DNA replication in human cells

**Shwetha Krishna**[1,2], **Aleš Obrdlík**[1], **Anton Zuev**[4], **Praveenkumar Rengaraj**[1,3], **Maria Zlobina**[1], **Helena Peschelová**[1,3], **Veronika Kozlová**[1], **Michal Šmida**[1], **Jana Dobrovolná**[4], **Štěpánka Vaňáčová**[1]

1. *Central European Institute of Technology, Masaryk University, Brno, Czech Republic*
2. *Faculty of Medicine, Masaryk University, Brno, Czech Republic*
3. *Faculty of Science, Masaryk University, Brno, Czech Republic*
4. *Institute of Medical Genetics, Czech Academy of Sciences, Prague, Czech Republic*

**E-mail of the presenting author:** shwetha.krishna@ceitec.muni.cz

N6-methyladenosine (m6A) and N6-2'-O-dimethyladenosine (m6Am) are two highly prevalent and dynamic eukaryotic mRNA modifications that exert regulatory effects on multiple steps of mRNA metabolism. m6A, in particular, has also been shown to regulate a vast majority of different cellular processes, including their newly identified roles in the maintenance of genome stability.

Here, we demonstrate that FTO, an RNA demethylase of m6A and m6Am, is a critical player in replication fork dynamics. Our omics studies reveal physical and genetic interactions between FTO and DNA replication factors. We identify that FTO is physically present at sites of active replication, and its demethylation activity is critical for normal replication fork progression. Under conditions of replication stress, FTO exercises a protective role over stalled replication forks to prevent the degradation of nascent DNA. Prolonged replication stress in the absence of FTO was seen to result in DNA strand breaks, possibly due to the collapse of stalled replication forks. Collectively, our results demonstrate a previously unknown role of FTO in the maintenance of replication fork integrity and provide a potential link between RNA modifications and DNA replication.

# Uncovering How ADAR1 Knockout Affects Hepatitis C Virus

**Martin Kubů**[1], **Kristina Roučová**[1], **Martin Pospíšek**[1], **Václav Vopálenský**[1]

*1. Laboratory of RNA Biochemistry, Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague 2 - Nové Město, 12844, Czech Republic*

**E-mail of the presenting author:** kubumart@natur.cuni.cz

The Hepatitis C Virus (HCV), a member of the Flaviviridae family, is the causative agent of a disease affecting tens of millions globally. Although cutting-edge direct-acting antivirals (DAAs) have proven highly efficacious in treatment, a protective vaccine against HCV remains elusive. Despite its single-stranded nature, the HCV genome contains numerous secondary RNA structures. These structures could act as substrates for innate immune RNA-binding proteins, including Adenosine Deaminase Acting on double-stranded RNA 1 (ADAR1).

Evidence suggests that ADAR1-mediated editing of dsRNA molecules hinders the detection of these viral components by cytoplasmic antiviral signaling pathways. These pathways are crucial for the production of interferons, which in turn induce ADAR1. Consequently, ADAR1 is understood to function as a modulator of innate immunity. Prior research investigating the interplay between HCV and ADAR1 has largely attributed an antiviral role to the ADAR1 enzyme during HCV infection.

To assess the functional role of ADAR1 in HCV replication, we established an ADAR1 knockout (KO) cell line derived from Huh7.5 hepatocellular carcinoma cells, using the pJFH1-pUC system for HCV production.

These KO cells, along with the wild-type line, were employed to plot the HCV replication curve and determine susceptibility to infection. We also used these cells to propagate infectious HCV particles and performed identical comparative experiments with the resulting viral stocks.

Our initial data suggest that knocking out the ADAR1 enzyme does not significantly impact the quantity of HCV RNA generated. However, we have observed that it does influence the vulnerability of Huh7.5 cells to HCV infection. Furthermore, we provide evidence that HCV appears to adapt over time to an environment lacking the ADAR1 enzyme.

*Acknowledgement*

*This research was supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union - Next Generation EU, by The Charles University Grant Agency (GAUK, no 249623) and by the Project P JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, Co-Funded by the European Union*

# Targeted antisense oligonucleotide therapy rescues PRPF31 expression in retinitis pigmentosa caused by a splicing mutation

**Poulami Banik[1], Felix Zimmann[1], Prasoon K Thakur[1], L'ubica Ďuďáková[5], Kateřina Večerková[3], Ondrej Kostov[4], Michal Kolář[3], Petra Lišková[6], Tomáš Bárta[2], David Staněk[1]**

*1. Laboratory of RNA biology, Institute of Molecular Genetics, CAS*
*2. Department of Histology and Embryology, Faculty of Medicine, Masaryk university, Brno, Czech Republic*
*3. Laboratory of Genomics and Bioinformatics, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic*
*4. University of Colorado, Boulder*
*5. Laboratory of the Biology and Pathology of the Eye, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic*
*6. Department of Ophthalmology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Prague, Czech Republic*

**E-mail of the presenting author:** poulami.banik@img.cas.cz

Retinitis pigmentosa (RP) is a genetic disorder leading to the progressive loss of photoreceptors and vision. Pre-mRNA processing factor 31 (PRPF31), a splicing factor, accounts for ~10% of autosomal dominant RP cases. We identified a novel intron 10 point mutation in PRPF31 that caused RP in two siblings of the same family. Splicing assays revealed that intron 10 is inefficiently removed even in wild-type samples. Using a splicing reporter, we found that the mutation further reduced proper splicing and activated a cryptic splice site, producing an aberrant transcript extended by 10 bp.Protein analyses from patient blood samples showed reduced expression of PRPF31 and other splicing proteins. Immunoprecipitation demonstrated that the mutant PRPF31 is unstable and interacts weakly with PRPF6, impairing tri-snRNP assembly. In-silico modeling and immunocytochemistry further revealed loss of a complete nuclear localization signal, leading to partial mislocalization.To correct splicing, we designed antisense oligonucleotides (ASOs) targeting exons 10–11 and intron 10. ASO treatment improved splicing in both wild-type and mutant contexts, including in patient-derived iPSCs and iPSC-derived retinal pigment epithelium (RPE) cells. Finally we have shown that ASO could improve the PRPF31 expression at protein level. This study identifies a novel PRPF31 mutation, characterizes its molecular consequences, and demonstrates that ASOs can rescue splicing defects, highlighting their therapeutic potential for RP.

# RNAs untangled: How RNA-binding proteins shape structure of long RNAs

**Lukáš Pekárek**[1,2,3], **Andreas Hartmann**[3], **Fiona Anilkumar**[1,2], **Fathima Hisana Ferosh**[1,2], **Simon Doll**[1,2], **Manthan Raj**[1,2], **Michael Schlierf**[3], **Marcus Jahnel**[1,2]

1. *Physics of Life (PoL), Cluster of Excellence, Technical University Dresden, Germany*
2. *BIOTEC - Biotechnology Center, Technical University Dresden, Germany*
3. *B CUBE – Center for Molecular Bioengineering, Technical University Dresden, Germany*

**E-mail of the presenting author:** Lukas.pekarek1@tu-dresden.de

RNA is an intriguing molecule. Despite its relatively simple composition, RNA's functional versatility underscores the crucial role of RNA structure. Proper folding enables distant segments of the RNA molecule to come into close proximity, facilitating essential biological functions. This is particularly critical for long RNAs such as mRNAs, rRNAs, and lncRNAs, which can span over 1000 nucleotides. The function of these RNAs depends critically on their structure and ability to cooperatively interact with RNA-binding proteins, which often contain intrinsically disordered regions prone to condensation.

**This raises a fundamental challenge: how do living organisms ensure the robust and accurate folding of long regulatory RNAs? Do biological condensates help to shape RNA structures? And conversely, how do regulatory RNAs influence the formation of these condensates?**

In this project, we aim to understand how long RNA molecules fold into their complex structures and what the role of RNA-binding proteins is. We took lncRNA HOTAIR and YBX1 RNA-binding protein as a case study to shed some light on this folding enigma. By employing methods like single-molecule optical tweezers, fluorescence correlation spectroscopy, or confocal microscopy, we want to understand the key aspects of the dynamic folding process of complex RNAs in the context of biomolecular condensates

# Primordial interactions between RNA and short peptides

**Simone Codispoti[1,2], Martin Mašek[1], Michal Kolář[1]**

1. *University of Chemistry and Technology, Prague, Czechia*
2. *University of Milan, Italy*

**E-mail of the presenting author:** kolarh@vscht.cz

The widely accepted *RNA world* theory has been challenged over the past decade. With others, we also argue that peptides played a more significant role in the primordial optimization of biomolecular complexes than previously recognized [1]. Nevertheless, our understanding of this ancient biomolecular relationship still contains many gaps. For example, it remains unclear how peptides influenced RNA stability. Similarly, the impact of early amino acid composition on peptide–RNA interactions is not yet understood.

The modern ribosome provides one of the best sources of information on the co-evolution of RNA and proteins. Drawing on the available literature and structural models of the ribosome, we constructed several computational models of so-called protoribosomes, putative predecessors of the modern ribosome, and performed all-atom molecular dynamics simulations. We characterized the conformational dynamics of these protoribosomes and proposed mechanisms by which they may have interacted with peptides of different compositions [2]. Our findings highlight the importance of considering peptides in models of early molecular evolution.

[1] Kolář, Hlouchová: The evolution of protein-RNA interactions, Curr. Opin. Struct. Biol. 2025.

[2] Codispoti et al.: The interplay between peptides and RNA is critical for protoribosome compartmentalization and stability, Nucl. Acids Res. 2024.

# Innovations in RNA sequencing: From Long Transcripts to Spatial omics

**Lea Bartoňová**

*Explorea s.r.o., Praha, Czech Republic*

**E-mail of the presenting author:**

RNA-seq is exceptionally versatile, and Takara Bio offers innovative kits to prepare RNA-seq libraries from a wide variety of starting materials for diverse goals. The presentation will introduce two recent innovations: the Long mRNA Seq Kit, enabling capturing of full-length transctipts, and Trekker/Seeker, a flexible solution for spatial gene expression mapping directly from tissue sections, including FFPE samples. We will highlight technical recommendations, insights from recent publications, and practical applications of these tools within standard RNA Seq workflows.

# From Recognition to Release: RNA Interactions with Mammalian Dicer

**Nikola Nosková[1,2], David Zapletal[1,2], Jan Petrovský[1,2], Petr Svoboda[3], Jiří Nováček[1], Richard Štefl[1,2]**

*1. CEITEC–Central European Institute of Technology, Masaryk University; Brno, Czechia*
*2. National Centre for Biomolecular Research, Faculty of Science, Masaryk University; Brno, Czechia*
*3. Institute of Molecular Genetics of the Czech Academy of Sciences, v.v.i., Prague 4, Czech Republic*

**E-mail of the presenting author:** nikola.noskova@ceitec.muni.cz

The microRNA (miRNA) pathway is a central mechanism of post-transcriptional gene regulation. In mammals, one of the key proteins in this pathway is the RNase III enzyme Dicer. Dicer processes precursor miRNAs (pre-miRNAs) into mature miRNAs that are subsequently loaded into Argonaute proteins. While many steps of the Dicer cycle have been structurally characterized, critical events such as the initial recognition of pre-miRNA and the release of mature miRNA to Argonaute remain poorly understood in mammalian Dicer. Moreover, unstructured regions of the protein have been largely overlooked, despite their emerging role in facilitating RNA release.

In this project, we aim to complete the structural and functional picture of the Dicer cycle by integrating cryo-electron microscopy with biochemical approaches. These studies will fill missing gaps in the mechanism, providing insight into how Dicer hands over miRNAs to Argonaute and contributes to the assembly of the RNA-induced silencing complex (RISC).

# Interplay between mitoribosome biogenesis and RNA editing in Trypanosoma brucei

**Prashant Chauhan[1], Alena Zíková[1], Ondřej Gahura[1]**

*1. Institute of Parasitology, Biology Centre CAS, Ceske Budejovice, Czech Republic*

**E-mail of the presenting author:** gahura@paru.cas.cz

Biogenesis of mitoribosomal small subunit (mtSSU) in *Trypanosoma brucei* involves three precursors characterized by cryoEM, containing together 39 assembly factors (AFs). The earliest intermediate, a ~4MDa complex called mtSSU assemblosome, features a tower-like protrusion formed by an essential homopentameric AF mt-SAF24, which is capped with a disc-shaped density of unknown composition. We genetically modified *T. brucei* to remove the N-terminal domain of mt-SAF24, resulting in the disconnection of the cap from the rest of the complex. Comparison of complexes with and without the cap by IP-MS using an assemblosome-specific AF mtSAF16 as a bait unambiguously revealed that the density is formed by a trimer of p22, a protein acting in uridine insertion/deletion RNA editing characteristic for kinetoplastid mitochondria. p22 interacts with multiple components of RNA editing substrate binding complex (RESC) and its downregulation by RNAi affects editing of mRNA of COII, a subunit of complex IV of OXPHOS system, but no other transcripts that require editing. Yet, the levels of all OXPHOS complexes with mitochondrial encoded subunits were markedly decreased upon p22 RNAi. We documented that the ablation of p22 results in reduced amounts of mtSSU and consequently impaired mitochondrial translation. Thus, p22 is an essential player in mtSSU assembly and represents an unprecedented physical link between RNA editing and mitoribosome biogenesis.

# Frequent occurrence and predicted functions of tRNAs with 4-base-pair anticodon stems in bacteria and phages: extended superwobble hypothesis

**Fadel Fakih**[1,2], **Satish Nandipati**[1,2], **Ambar Kachale**[1,2], **Jiří Heller**[1], **Jakub Žváček**[5], **Zdeněk Paris**[1], **Leoš Shivaya Valášek**[3], **Pragya Tripathi**[1], **Michal H. Kolář**[5], **Vyacheslav Yurchenko**[4], **Marek Eliáš**[4], **Eugene V. Koonin**[6], **Julius Lukeš**[1,2], **Anzhelika Butenko**[1,2,4]

*1. Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Budweis), Czech Republic*
*2. Faculty of Sciences, University of South Bohemia, České Budějovice (Budweis), Czech Republic*
*3. Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*
*4. Department of Biology and Ecology, Faculty of Science, University of Ostrava, Ostrava, Czech Republic*
*5. Department of physical chemistry, University of Chemistry and Technology, Technicka 5, 16628 Prague, Czech Republic*
*6. National Center for Biotechnology Information, National Institutes of Health, Bethesda, USA*

**E-mail of the presenting author:** fadel.fakih@paru.cas.cz

Codon reassignment, the redefinition of a codon to encode a different amino acid, is a powerful evolutionary force that does affect translation efficiency and shape the proteome composition. We recently described in the trypanosomatid protist *Blastocrithidia nonstop* a novel mechanism for UGA-to-tryptophan reassignment involving a 4-base-pair (bp) anticodon stem (AS) tRNA-Trp-CCA, a structural deviation from the canonical 5-bp AS tRNA (Kachale et al., Nature 2023). The shortening of the AS enables C:A pairing at the third position of the codon extending the decoding flexibility of the tRNA. To determine whether this mechanism is unique to eukaryotes or evolved also in other domains of life, we analyzed 42,109 bacterial and 733 phage genomes, revealing frequent presence of 4-bp AS tRNA-Trps, and that their occurrence strongly correlates with the UGA-to-tryptophan reassignment. The genome of the endosymbiotic bacterium Candidatus *Zinderia insecticola* and that of a Jade phage with UGA-to-tryptophan reassignment contain only the near-cognate 4-bp AS tRNA-Trp-CCA, lack both canonical 5-bp AS tRNA-Trp-CCA and a suppressor tRNA fully cognate to UGA. The secondary structural analysis shows high similarity between these bacterial, phage, and trypanosomatid tRNAs, suggesting convergent evolution of this solution across the domains of life. We tested several predictions based on incorporating previously excluded possibility of C:A pairing at the 3rd codon position, and conclude that the expanded codon recognition capacity might apply to other tRNA molecules beyond tRNA-Trp-CCA. We experimentally demonstrated the UGA readthrough capacity of the 4-bp AS tRNA-Trp-CCA in *Escherichia coli* and complementary atomistic molecular dynamics simulations offered a possible mechanistic explanation. These findings expand the toolbox of functional tRNA structures and provide new insights onto translational flexibility and the evolution of the genetic code.

# Deciphering the translation mechanism in Blastocrithidia nonstop: a trypanosomatid with all three stop codons reassigned

**Nathalia Ballesteros**[1,2], **Jiri Koubek**[1], **Julie Kovářová**[1], **Zdeněk Paris**[1]

1. *Institute of parasitology, Biology Centre CAS, České Budějovice, Czech Republic*
2. *Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

**E-mail of the presenting author:** nathalia.ballesteros@paru.cas.cz

*Blastocrithidia nonstop* is a trypanosomatid in which all three stop codons are reassigned to encode amino acids. Previous findings have uncovered the key molecular mechanisms behind this stop codon readthrough (SC-RT), including the presence of novel cognate tRNAsGlu, a shortened anticodon stem in the canonical tRNATrp, and specific mutations in release factors that facilitate SC-RT (Kachale et al., *Nature* 2023).

Despite these discoveries, several fundamental questions remain regarding the impact of these alterations on translation efficiency and termination. Ribosome profiling (RiboSeq), a technique based on deep sequencing of ribosome-protected fragments (RPFs), provides a powerful tool to study translation dynamics. Thus, we standardized the RiboSeq approach in *B. nonstop* and observed that while most proteins terminate at the canonical stop codon, hundreds of cases exhibit C-terminal extensions beyond the canonical UAA stop codon.

As previously hypothesized, an overrepresentation of the UAA in 3' UTRs could play a critical role in proper translation termination in *B. nonstop*. Our RiboSeq data support this, revealing an apparent correlation between read density in the extended region and the proximity and frequency of additional in-frame UAA codons. These initial findings provide new insights into the broader translational implications of SC-RT, guiding further exploration of the mechanisms behind this novel genetic code variation.

# Unveiling the silence: how translation quiescence shapes mRNA stability in oocytes

**Fatima J. Berro**[1,2], **Michal Dvoran**[1], **Andrej Susor**[1]

*1. Laboratory of Biochemistry and Molecular Biology of Germ Cells, IAPG CAS, Rumburská 89, 277 21 Liběchov, Czech Republic*
*2. Department of Developmental and Cell Biology, Faculty of Sciences, Charles University, Prague, Czech Republic*

**E-mail of the presenting author:** berro@iapg.cas.cz

During oogenesis and embryogenesis, intricate processes precisely regulate gene expression to accumulate maternal mRNAs that are important for oocyte physiology and early embryonic development before global transcription is silenced. When transcription is silenced, post-transcriptional regulation becomes the major mode of gene expression control, affecting the localization and stability of mRNA and its connection to the translational machinery.

mRNA translation is an extensively regulated process, and in the transcriptionally silent oocyte and early embryo, translation is neither continuous nor uniform but rather exhibits ''oscillatory patterns'' that coincide with specific stages of oocyte maturation and embryonic development. Given this fact and the fact that translation of maternal mRNAs is activated at specific developmental milestones, several questions arise about the fate of mRNAs during ON-OFF periods of translation. Our hypothesis assumes that translational quiescence leads to mRNA stabilization. Methods such as RNA fluorescence in situ hybridization (RNAFISH) and RNA-puromycin proximity ligation assay (RNA-PuroPLA) are used to explore the dynamic interplay between mRNA stability and translation by visualizing and quantifying mRNA molecules and their translation in situ in control oocytes compared to oocytes treated with translation inhibitors with different mechanisms of action. Our results show that the effect of translation inhibition on mRNA stability mainly depends on the mode of translation inhibition and whether this mRNA is actively translated or dormant under physiological conditions. A large part of the work will focus on the localization of mRNA, the mechanistic investigation of how translational quiescence leads to changes in mRNA stability, and how this affects oocyte developmental competence and embryogenesis.

# All About A's: The distinct roles of eIF4A1 and eIF4A2 in T cell translation during activation

**Adriana Šubrtová**[1]

*1. Institute of Microbiology, CAS*

**E-mail of the presenting author:** adriana.subrtova@biomed.cas.cz

T cells are key regulators of adaptive immunity and require precise control of protein synthesis during activation. The mechanism that controls the initiation of translation after their activation is still poorly understood. Eukaryotic initiation factors (eIFs), which regulate the initiation step of translation, play a central role in this process. eIF4A2 belongs to the DEAD-box RNA helicase family, which unfolds RNA structures to enable translation. It is closely related to eIF4A1. Despite its potential importance, eIF4A2 remains largely misunderstood. While eIF4A1 is a well-known essential helicase required for active translation, new evidence suggests that eIF4A2 may have a different regulatory function. It is possible that eIF4A2 modulates translation in a in a cell type and activation state – dependent manner. Understanding the function of eIF4A2 is critical because its dysregulation has been linked to tumor progression and immune disorders. This makes eIF4A2 a promising target for elucidating the mechanisms that control translation during T cell activation and related diseases.

# Few products, dozens of applications

**Jakub Dušek**

*PentaGen s.r.o., Kladno, Czech Republic*

**E-mail of the presenting author:** jdusek@pentagen.cz

During the lecture, proven as well as completely unique solutions in RNA sequencing will be presented. These solutions cover the areas of bulk transcriptomics, single-cell transcriptomics, and spatial transcriptomics. Whether you are interested in bacteria, yeast, flowers, fishies, rats, or people, PentaGen has a solution to help you on your exciting research journey!

# Posters

## 1
## No nonsense allowed!

**Nawal Al-Chamy**[1], **Filip Brázdovič**[1], **Leoš Valášek**[1]

*1. Laboratory of regulation of gene expression , Institute of Microbiology of the Czech Academy of Sciences, Czech Republic*

**E-mail of the presenting author:** nawal.alchamy@biomed.cas.cz

Normally, translation ends when one of three termination codons (UAA, UAG, UGA) is recognized by a release factor at the end of the coding mRNA sequence, prompting the ribosome to release a complete, functional protein. However, when a mutation occurs and introduces a premature termination codon (PTC), the affected mRNA is either targeted for degradation by the nonsense-mediated decay pathway or the PTC causes premature translation termination resulting in a truncated and thus usually nonfunctional protein. Notably, these nonsense mutations account for approximately 11% of all genetic diseases.

Nature offers rare examples of organisms with reassigned termination codons. One such organism is *Blastocrithidia nonstop* in which the UAG and UAA codons were reassigned to glutamic acid by acquiring new tRNAGlu genes with anticodons fully matching to the termination codons. As a result, all the in-frame UAR codons are efficiently decoded as Glu.

The UGA reassignment, this time to tryptophan, occurred in a much more intriguing way. A relatively minor change in the structure of the *B. nonstop* tRNATrp, specifically the shortening of its anticodon stem (AS) from the canonical 5 bp to 4 bp, greatly enhanced its ability to wobble base pair with and thus read through the in-frame UGA codons.

Inspired by this evolutionary novelty, our project investigates whether similar structural alterations of all existing near-cognate tRNAs can enhance stop codon readthrough. We observed varying effects among the tested near-cognate tRNAs such as that some showed increased readthrough when their AS was shortened. Some of these mutated tRNAs displayed a further enhancement when their anticodon was mutated to make it fully cognate to the corresponding stop codon, while others showed no change or even a negative effect. These findings point at specific near-cognate tRNAs that might have a potential to be engineered to become potent PTC suppressors by introducing relatively simple changes. Further mechanistic and structural insights are needed to understand the underlying principle of these observations that will be discussed.

# 2
# Global analysis of the Hfq-mediated RNA interactome discovers a MicA homolog in Bordetella pertussis

**Martin Beles**[1], **Argha Saha**[1], **Branislav Večerek**[1]

*1. Institute of Microbiology of Czech Academy of Sciences*

**E-mail of the presenting author:** martinbeles1996@gmail.com

*Bordetella pertussis* is a causative agent of whooping cough, a highly contagious respiratory disease. For the successful transmission and host colonization, the pathogen uses a wide range of virulence factors and strategies. These processes require strictly regulated gene expression and recent studies show that small RNAs may play a key role in this regulation. Here we analysed roles of a sRNA candidate transcript (CT) 532. CT_532 is transcribed from intergenic region between gene *BP3441* and *pyrD* and we identified plausible promoter for this sRNA. To validate this outcome, we performed TEX-nuclease treatment of a total RNA sample, and our Northern blot analysis showed, that CT_532 is not degraded by TEX and thus may represent a primary transcript. We also analysed the expression of CT_532 in different mutant strains and Northern blot analysis showed that expression of CT_532 is strongly downregulated in the absence of Hfq chaperone, however in the absence of RNase III (*rnc*Δ85) the amount of the sRNA was significantly higher. Our RIL-seq indicated that CT_532 interacts with three mRNAs encoding outer membrane proteins (OMPs), namely BP0840, OmpA and Bph3. We also determined the homology of CT_532 with known OMPs regulators and found out that CT_532 shares 60% homology with *E. coli* sRNA MicA. We also analysed the expression of *ompA* in *Δhfq* a *rnc*Δ85 with RT-qPCR which revealed that the amount of full-length transcript is significantly increased in these mutants while the quantity of its shorter form was not changed. Since MicA was found to expressed under stressed conditions, we also analysed the expression of CT_532 under heat or cold shock and osmotic stress. The expression of the sRNA was upregulated during temperature shock, while the osmotic stress caused its downregulation compared to wt strain. These results indicate that CT_532 is indeed involved in regulation of the cell response to membrane stress. Together with this, we also analysed the expression of CT_532 in Δ*rseA* strain, since RseA is an anti-sigma factor to σE, the major transcription factor during membrane stress. However, the analysis did not show any significant change in the expression.

# 3
# Connection between the bacterial phosphotransferase system and antibiotic resistance

**ŠÁRKA BOBKOVÁ**[1], **Tamara Knežova Balgová**[1], **Petra Sudzinova**[1], **Alena Křenková**[2], **Karel Harant**[2], **Libor Krásný**[1], **Jana Wiedermannová**[1]

*1. Institute of Microbiology, Czech Academy of Science, Prague, Czech republic*
*2. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science, Prague, Czech republic*

**E-mail of the presenting author:** sarka.bobkova@biomed.cas.cz

The bacterial phosphotransferase system (PTS) is one of the three main transport mechanisms used by bacteria for the uptake of sugars and sugar derivatives. PTS is a cascade of proteins that transfer the phosphoryl group from phosphoenolpyruvate (PEP) to the transported substrate, resulting in simultaneous transport and phosphorylation of the substrate. The PTS cascade comprises membrane-associated sugar-specific proteins (EIIABCD), and cytoplasmic proteins (EI, HPr) shared across substrates. Unlike other transport systems, PTS functions not only as a transporter but also as a regulator of different physiological processes like carbon catabolite repression, sugar metabolism and sporulation. The principal regulatory component of the PTS is the cytoplasmic protein HPr, which exists in three states in the cell: unphosphorylated, phosphorylated on His 15 residue, or phosphorylated on Ser 46 residue. Histidine phosphorylated HPr mediates phosphate transfer from protein EI to membrane components of PTS, facilitating sugar transport. On the other hand, the serine phosphorylated HPr interacts with the carbon catabolite protein A (CcpA) to regulate gene expression, both positively and negatively. Although HPr-CcpA regulon is extensive, it has not yet been studied in connection with antibiotic resistance.

We show that in the Gram-positive *Bacillus subtilis,* the serine-phosphorylated HPr plays a role in the bacterial defence response to the antibiotic rifampicin. This role appears to be indirect, likely involving regulation of gene expression by serine-phosphorylated HPr in complex with CcpA. To investigate this regulatory mechanism at multiple molecular levels: protein-DNA, protein-RNA and protein-protein, we prepared samples for ChlpSeq, RNAseq and immunoprecipitation (IP) experiments. The preliminary results obtained from these experiments will be presented and discussed.

# 4
# Expanding the Mycobacterial Transcriptional Network: Discovery of UCPs

**Nabajyoti Borah[1], Hana Šanderová[1], Tomáš Koval[2], Alena Křenková[3], Martin Hubálek[3], Jan Dohnálek[2], Libor Krásný[1]**

*1. Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic*
*2. Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Průmyslová 595, 252 50, Vestec, Czech Republic*
*3. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 166 00 Prague, Czech Republic*

**E-mail of the presenting author:** nabajyoti.borah@biomed.cas.cz

Transcription regulation in bacteria depends on the coordinated activity of DNA-dependent RNA polymerase (RNAP) and associated transcription factors. While our understanding of bacterial transcription is largely based on model organisms such as *Escherichia coli* and *Bacillus subtilis*, species-specific transcriptional adaptations remain poorly understood. Mycobacteria, for instance, employ unique transcription factors such as CarD and RbpA- absent in *E. coli* - to modulate their gene expression. Another such mycobacterial factor, HelD (class II), recycles stalled RNAP by dissociating it from nucleic acids. Our previous work has shown that HelD also protects RNAP from the antibiotic rifampicin during transcription initiation. Here, using *Mycobacterium smegmatis* as a non-pathogenic model for *M. tuberculosis*, we identified several previously uncharacterized RNAP-associated proteins (UCPs). Among these, UCP1, which coexists with HelD, is essential for viability, suggesting its critical role in bacterial survival. Another RNAP-binding partner, UCP7, associates with membrane components, suggesting its role in cell envelope biogenesis or maintenance. Together, these findings expand the mycobacterial transcriptional network and uncover novel factors critical for gene expression and survival.

**Keywords:** Transcription factor; Uncharacterized protein; RNA polymerase; Mycobacteria

This project was supported by the Grant Agency of the Czech Republic; GAČR 210379, and the Grant Agency of Charles University in Prague; GA UK 236823.

# 5
# Directed Evolution of tRNA Molecules in Yeast

**Filip Brázdovič**[1], **Nawal Al-Chamy**[1], **Leoš Valášek**[1]

*1. Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic*

**E-mail of the presenting author:** filip.brazdovic@biomed.cas.cz

The genetic code is a set of rules that describe the process of translation of genetic information into an amino acid chain. The main players are aminoacyl-tRNA molecules and a ribosome, which together with translation factors ensure accurate translation of the mRNAs. In the standard genetic code, 61 of 64 codons specify one of 20 amino acids, while three codons (UAA, UAG, UGA) signal the end of translation. tRNA molecules are evolutionarily optimized to specifically decode their cognate and, where applicable, related "wobble" codons, while others are avoided. The primary discriminating factor is the codon-anticodon base-pairing between aminoacyl-tRNA and mRNA molecules, respectively the optimal geometry of the minihelix that they form. However, previous observations have indicated that other parts of the tRNA molecule, such as the anticodon loop and the anticodon stem, are also important for proper decoding. This can be illustrated by suppressor tRNAs that are fully cognate to one of the termination codons. While some are highly effective, others are unable to significantly increase readthrough of termination codons without additional changes in their tRNA. Normally, there are no tRNA molecules in cells dedicated to decoding termination codons. However, there are organisms with alternative genetic codes that have converted some or even all the termination codons into sense codons. To achieve this, their entire translational machinery has had to adapt. These adaptations include the emergence of novel tRNA molecules that are particularly suited for this task. Inspired by this, our goal is to evolve specific suppressor tRNA molecules *in vivo*, by employing CRISPR-guided Cas9 nickase fused to an error-prone DNA polymerase (EvolvR), that will be able to efficiently read-through given termination codons without a negative effect on the cell fitness. The progress of this evolution-in-a-test-tube task will be discussed.

# 6
# Uncovering the Assembly of the RISC-Loading Complex

**Nikola Nosková[1,2], David Zapletal[1,2], David Buchta[1,2], Jan Petrovský[1,2], Karel Kubíček[1,2], Richard Štefl[1,2]**

*1. CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czechia*
*2. National Centre for Biomolecular Research, Faculty of science, Masaryk University Brno, Czechia*

**E-mail of the presenting author:** David.Buchta@ceitec.muni.cz

Argonaute and Dicer proteins are key components of RNA-dependent gene silencing. In mammals, Argonaute-2 (Ago2) functions as the primary effector of microRNA (miRNA)-mediated gene regulation. Following Dicer-mediated processing of precursor miRNAs (pre-miRNAs), Ago2 is selectively loaded with mature miRNA guides to form the RNA-induced silencing complex (RISC). Despite more than two decades of extensive research, the molecular rules and structural basis governing guide-strand selection and loading remain only partially understood. Notably, multiple studies suggest that Dicer and its cofactor TARBP2 (or PACT) play important roles in facilitating the accurate and efficient loading of Ago2. Here, we present our preliminary efforts to characterize the molecular interactions between Ago2 and the Dicer–TARBP2 complex, as well as the specific contributions of each component during the RISC-loading process. Our structural biology approach provides insights into the roles of the individual components and sheds light on the overall mechanism of RISC assembly.

# 7
# Role of eIF3 Subunit Imbalance in Modulating Translation Under Intestinal Inflammatory Conditions

**Anna Smirnova[1], Štěpán Coufal[2], Zuzana Jirásková Zákostelská[2], Miloslav Kverka[2], Leoš Shivaya Valášek[1]**

*1. Laboratory of Regulation of Gene Expression, The Institute of Microbiology of the Czech Academy of Sciences, Prague, the Czech Republic*
*2. Laboratory of Cellular and Molecular Immunology, The Institute of Microbiology of the Czech Academy of Sciences, Prague, the Czech Republic*

**E-mail of the presenting author:** coufal.st@gmail.com

Protein translation is a fundamental biological process that demands a lot of energy in the cell. In eukaryotes, translation is tightly regulated at both general and gene-specific levels. General regulation can be mediated by translation-related factors known as eukaryotic initiation factors (eIFs), among which the multisubunit eIF3 plays a crucial role.

Recent studies indicate that inadequate regulation of protein synthesis and improperly balanced eIF3 expression is not only a hallmark of cell transformation and cancer but also contributes to other human diseases such as Inflammatory Bowel Disease (IBD). Here we investigated the molecular mechanisms of eIF3-mediated translational control involved in the pathogenesis of IBD (i.e., ulcerative colitis (UC) and Crohn's disease).

To gain deeper mechanistic insights and model key aspects of human UC, we employed a mouse model of acute dextran sulfate sodium (DSS; MP Biomedicals, CA, USA) induced colitis, which faithfully mimics several histopathological and immunological features of the human disease.

This model provides a valuable experimental platform for studying disease progression and for exploring translational regulatory pathways that may contribute to intestinal inflammatory conditions in IBD. As a next step, ribosome profiling (Ribo-seq) is planned to globally assess translational activity and uncover transcript-specific regulation of translation in colitis. We further propose that the IBD-provoked switch in the expression profile of target mRNAs may be facilitated by eIF3. Planned Ribo-seq is expected to contribute to a more comprehensive understanding of the molecular basis of IBD.

This research was supported by The Czech Science Foundation (GAČR) grant No. 24-10013S and Czech health research council (AZV ČR) grant No. NU22-07-00216

# 8
# NOVEL ERYTHROMYCIN RESISTANCE MECHANISM MEDIATED BY MrmA METHYLTRANSFERASE IN C. DIFFICILE

**Fanny DEMAY[1], Karel SKUBNIK[2], Vojtech KOVAŘOVIC[1], Michaela NOVOTNÁ[1], Martin HLAVATY[3], Annemieke FRIGGEN[3], Weis K. SMITS[3], Marcela KRŮTOVÁ[4], Gabriela BALÍKOVÁ NOVOTNÁ[1]**

*1. Institute of Microbiology, The Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic*
*2. CryoElectron Microscopy and Tomography Core Facility, Central European Institute of Technology, Brno, 601 77, Czech Republic*
*3. Experimental Bacteriology, Leiden University Center for Infectious Diseases, Leiden Medical Center, Leiden, Netherlands*
*4. Department of Medical Microbiology, Charles University 2nd Faculty of Medicine and Motol University Hospital, Prague, Czech Republic*

**E-mail of the presenting author:** fanny.demay@biomed.cas.cz

*Clostridioides difficile* is a leading cause of hospital-acquired diarrhea. However, the rise of antimicrobial resistance in epidemic *C. difficile* lineages compromises treatment efficacy and increases the risk of spreading resistance to other pathogens.
We have identified a novel macrolide resistance gene, *mrmA* (macrolide resistance methyltransferase A), by comparative genomic analysis of erythromycin-susceptible and resistant *C. difficile* strains. The encoded MrmA, a putative SAM-radical 23S rRNA methyltransferase, shares homology with RlmN and Cfr. RlmN methylates the C2 atom at nucleotide A2503, which controls translational accuracy, whereas Cfr methylates the C8 atom at the same nucleotide, resulting in resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A, but not to erythromycin. However, heterologous expression of *mrmA* in *E. coli* resulted only in specific resistance to erythromycin and streptogramin B.

We hypothesize that MrmA, similar to RlmN or Cfr, possesses methyltransferase activity but targets a distinct adenine position, disrupting macrolide and streptogramin B binding. Direct 23S rRNA nanopore sequencing and primer extension assays have indeed revealed altered methylation patterns at the 23S rRNA nucleotide A2058 in strains producing MrmA. This nucleotide is typically dimethylated at its N6 atom by Erm-family methyltransferases, conferring broad resistance to macrolides, lincosamides, and streptogramin B antibiotics. Structural basis of MrmA-mediated ribosome modification was determine by Cryo-EM studies and reveal new methylation on C2 of A2058.

This research uncovers a unique resistance mechanism mediated by RlmN-family enzymes in *C. difficile*, providing new insights into their substrate adaptability and the evolution of antibiotic resistance.

Acknowledgement : The work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – NextGenerationEU.

# 9
# Exploring the development and application of catalytic DNA

**Martin Volek[1], Kateřina Švehlová[1], Jaroslav Kurfürst[1,3], Martin Jakubec[1,2], Patrik Lettrich[1,2], Zuzana Kráľová[1,2], Karolína Pšenáková[1], Kristýna Patková[1,2], Tereza Šrámková[1], Václav Veverka[1,2], Kateřina Dušková[1], Edward Arthur Curtis[1]**

1. *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences*
2. *Faculty of Science, Charles University*
3. *University of Chemistry and Technology Prague*

**E-mail of the presenting author:** katerina.duskova@uochb.cas.cz

The DNA double helix is perfectly suited for storing genetic information, but its potential goes far beyond this canonical role. In our group, we explore the broader functional possibilities of DNA using a strategy called artificial evolution. This method allows us to isolate rare molecules with valuable properties - such as the ability to bind specific ligands or catalyze reactions - from enormous libraries of random sequences.

Here, we present an overview of DNA enzymes (DNAzymes) recently discovered in our lab that produce chemiluminescent, fluorescent, and colorimetric signals. These DNAzymes offer a simple and rapid workflow, can be engineered to respond selectively to specific inputs, and hold promise for applications such as high-throughput screening. We also describe new approaches for probing sequence space through the design of structured libraries and single-step selection strategies. Together, our work demonstrates the power of catalytic DNA and highlights how it can be harnessed to address real-world challenges.

*Acknowledgment: This work was supported by OP JAK (RNA4T grant No. CZ.02.01.01/00/22_008/0004575), GAČR (grant No. 2411134S, 2411210S) and GA UK (projec No. 416425).*

# 10
# Structural insights into how bacteria regulate the expression of carbon-catabolism enzymes

**Adéla Fejfarová**[1,2], **Markéta Šoltysová**[1], **Irena Sieglová**[1], **Milan Fábry**[1], **Jana Škerlová**[1], **Pavlína Maloy Řezáčová**[1]

*1. Laboratory of Structural Biology, Institute of Organic Chemistry and Biochemistry, Prague, Czechia*
*2. Department of Cell Biology, Faculty of Science, Charles University, Prague, Czechia*

**E-mail of the presenting author:** adela.fejfarova@uochb.cas.cz

The process of gene expression comprises number of steps, including transcription of DNA into RNA, translation of RNA into protein, and protein modifications. In bacteria, the regulation of the expression pathway represents the major answer to any environmental changes, therefore a critical adaption for cell survival. Based on the availability of different nutrients, bacteria turns on and off different genes encoding for proteins of the metabolic pathways. The whole metabolic machinery can be regulated at the very beginning of the expression, namely the initiation of transcription. For that, diverse bacterial groups use transcription repressors of the GntR family. The members of the GntR family are mainly responsible for regulation of enzymes utilizing catabolism of various carbon sources (e.g. sugars). The family is defined by a conserved N-terminal DNA-binding domain (DBD) with winged-helix-turn-helix motif. The C-terminal effector binding domain (EBD) distinguishes these regulators into five subfamilies. When an effector binds the EBD, the transcription repressor loses its ability to bind DNA, which consequently leads to activation of transcription. Although there are thousands of these repressors across most bacterial species, their mechanism of action is hardly ever understood on the structural level. Here we present the initial structural biology study on the prototypic member GntR, from the model organism of gram-positive bacteria *Bacillus subtilis*. By the method of cryogenic electron microscopy (cryoEM), we try to understand the molecular mechanism behind the protein-DNA complex formation, what are the spatial arrangements, and how these change upon effector binding.

# 11
# High-throughput screening for enhancing PRPF31 expression in retinitis pigmentosa model

**Ivana Halova**[1], **Viktor Sinica**[2], **Petr Bartunek**[2], **David Stanek**[1]

*1. Laboratory of RNA Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic*
*2. CZ-OPENSCREEN, National Infrastructure for Chemical Biology, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic*

**E-mail of the presenting author:** ivana.halova@img.cas.cz

Retinitis pigmentosa (RP) is the most common inherited retinal disease, characterized by the progressive degeneration of photoreceptors. Patients with RP experience night blindness, and the disease can eventually lead to complete loss of vision. Among the proteins linked to RP, splicing factors such as *PRPF31* are particularly notable. Mutations in *PRPF31* account for nearly 10% of autosomal dominant RP cases, often abrogating expression of the PRPF31 protein from the mutated allele and leading to haploinsufficiency. Remarkably, the incomplete penetrance observed in PRPF31-linked RP (RP11) highlights the critical role of a sufficient amount of functional PRPF31 protein expressed from the wild-type allele in the retina. Currently, no cure exists for PRPF31-dependent RP, although vitamins and nutritional supplements may slow its progression.

In this study, we propose and validate a high-throughput screening method based on an EGFP-fluorescence readout to quantify PRPF31 expression levels following treatment with clinically relevant drug libraries. To this end, we established an RPE cell line in which the PRPF31 protein was tagged with EGFP at one allele (heterozygous, Hz) or at both alleles (homozygous, Ho). Changes in EGFP fluorescence of PRPF31-Hz cells were evaluated 24 hours post-treatment, while PRPF31-Ho cells served as positive controls. Promising hits were further analyzed by flow cytometry and western blotting to optimize drug concentrations and to validate the high-throughput screening results. Selected drugs that increased PRPF31 expression without compromising cell viability were tested for their ability to enhance PRPF31 expression in patient-derived RPE cells and retinal organoids.

We believe that our proposed system will facilitate the identification of new drugs capable of positively influencing PRPF31 expression, potentially leading to novel therapeutic strategies for RP.

# 12
# EIF3-mediated translational control of MAPK/ERK signaling pathway

**Anna Herrmannová**[1], **Jan Jelínek**[2], **Klára Pospíšilová**[1], **Leoš Valášek**[1]

*1. Laboratory of Regulation of Gene Expression, Institute of Microbiology of the Czech Academy of Sciences, Prague, Vídeňská 1083, 142 20, Czech Republic*
*2. Laboratory of Bioinformatics, Institute of Microbiology of the Czech Academy of Sciences, Prague, Vídeňská 1083, 142 20, Czech Republic*

**E-mail of the presenting author:** herrmannova@seznam.cz

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 translation in general, remain unclear. We have recently analyzed the impact of eIF3d, eIF3e or eIF3h depletion by siRNA, perturbing the eIF3 holocomplex stoichiometry, on the translatome of HeLa cells using Ribosome profiling (Ribo-seq) (Herrmannova et al. 2024). Depletion of eIF3d or eIF3e but not eIF3h reduced the levels of multiple components of the MAPK/ERK signaling pathways. Surprisingly, however, depletion of all three eIF3 subunits increased MAPK/ERK pathway activity, as observed by increased phosphorylation of ERK kinase and transcription factor c-Jun. Besides these published results, we also detected increased phosphorylation of RPS6 in the eIF3dKD. To address what downstream kinases are responsible for c-Jun and RPS6 phosphorylation, in other words, whether the mTOR or MAPK/ERK pathway, or both, are involved and how, we will use various kinase- and phosphatase-specific inhibitors (for example the MEK1/2 inhibitor PD0325901 abolishing ERK1/2 phosphorylation) and monitor their effects in wild-type (wt) vs. eIF3 KD strains.

# 13
# The effect of DNA topology on transcription dependent on the alternative SigN factor in Bacillus subtilis

**Kristýna Hladíková**[1], **Petra Sudzinová**[1], **Milada Kambová**[1], **Hana Šanderová**[1], **Libor Krásný**[1]

*1. Institute of Microbiology, The Czech Academy of Sciences, Vídeňská 1083, 142 00 Prague, Czech Republic*

**E-mail of the presenting author:** kristyna.hladikova@biomed.cas.cz

DNA topology plays a key role in the regulation of gene expression by affecting interactions between DNA and RNA polymerase (RNAP) and other regulatory proteins. A key characteristic of DNA topology is the level of supercoiling. A previous study showed that DNA relaxation decreases transcription from promoters dependent on main (σA) and alternative (σB, σD, σE, σF, σH) sigma factors from *Bacillus subtilis*. Surprisingly, there was one exception, the σN factor, where the trend is the opposite. The activity of RNAP on relaxed σN-dependent promoters was higher than on their supercoiled versions. The determinants of this behaviour were unknown. Here, we demonstrate that this property of σN-dependent promoter *sigN3* is not encoded by the core promoter sequence. Furthermore, we identified that the sequence causing a relatively high promoter activity on relaxed DNA lies approximately 20 bp upstream from the promoter *sigN3*. Finally, we tested another σN-dependent promoter *zpbY.* Its core promoter sequence showed high activity on relaxed templates. In future research we will focus on a better characterization of the promoter *sigN3* upstream sequence and on identifying the key differences between the two promoters.

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

# 14
# Expression, purification and initial functional characterization of DEAD-box RNA helicase Gemin3

**Veronika Holotová**[1], **David Staněk**[1]

*1. Institute of Molecular Genetics of the Czech Academy of Sciences*

**E-mail of the presenting author:** veronika.holotova@img.cas.cz

Gemin3 (also known as DDX20) is a conserved member of the DEAD-box family of RNA helicases, a class of enzymes that utilize ATP hydrolysis to remodel RNA structures and RNA–protein complexes. As a core component of the survival motor neuron (SMN) complex, Gemin3 plays an essential role in the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). Beyond its role in snRNP biogenesis, Gemin3 has been also implicated in diverse aspects of RNA metabolism, including micro RNA processing, transcriptional regulation, and ribonucleoprotein trafficking. Despite its central role in RNA metabolism, the molecular mechanisms underlying Gemin3's helicase and ATPase activities remain poorly defined, and direct biochemical evidence for its RNA remodeling capacity is limited. In this work, we report the successful expression and purification of the helicase core of the human Gemin3 and provide its inital biochemical characterization. Using a fluorescence resonance energy transfer (FRET) based molecular beacon assay, we monitored RNA unwinding activity on a model substrate derived from human U2 snRNA sequence. Although our data are preliminary, these experiments demonstrate the feasibility of dissecting Gemin3 helicase function *in vitro*. Our ongoing studies aim to extend these initial observations toward a more detailed mechanistic understanding of how Gemin3 facilitates RNA remodeling.

# 15
# The position of δ on RNA polymerase and the function of its C-terminal domain

**Klára Juříková Mikesková[1,2], Hana Šanderová[1], Martin Černý[3,4], Markus Wahl[5], Libor Krásný[1]**

*1. Department of Microbial Genetics and Gene Expression, Institute of Microbiology, Czech Academy of Sciences, Prague CZ-14220, Czech Republic*
*2. Faculty of Science Charles University, Albertov 6, Prague 2 12800, Czech Republic*
*3. National Centre for Biomolecular Research, Masaryk University, Kamenice 5, Brno 62500, Czech Republic*
*4. Central European Institute of Technology, Masaryk University, Kamenice 5, Brno 62500, Czech Republic*
*5. Laboratory of Structural Biochemistry, Institute of Chemistry and Biochemistry, Freie Universität Berlin, Takustr. 6, D-14195 Berlin, Germany*

**E-mail of the presenting author:** klara.mikeskova@biomed.cas.cz

δ is a small subunit of bacterial RNA polymerase (RNAP) in Gram-positive bacteria. It consists of a structured N-terminal domain (δNTD) and a highly acidic and unstructured C-terminal domain (δCTD). The position of δ on RNAP and its function are still a subject to debate. Here we first present a Cryo-EM structure of a complex of the holoenzyme with DNA, showing the position of the δNTD on RNAP. Second, we address how the δCTD affects transcription. Using δ subunits of *Bacillus subtilis* and *Staphylococcus aureus* we created chimeric and C-terminally shortened protein variants. Our experiments revealed that *S. aureus* δCTD was unable to substitute *B. subtilis* δCTD. The δCTDs thus appear to be species-specific, despite both being highly acidic and unstructured. Furthermore, shortening the *B. subtilis* δ from the C-terminus negatively affected the competitive fitness of the cells. This project thus expands our understanding of the architecture of RNAP and the function of its δ subunit in Gram-positive bacteria.

This work was supported by the Czech Science Foundation grant No. 22-06342K.

# 16
# 3 to 1 Initiation Tango: eIF3's Choreography in Human AUG Recognition

**Pragya Kamal**[1], **Filip Trčka**[1], **Terezie Prilepskaja**[1], **Leoš Valášek**[1]

*1. Laboratory of gene regulation, Institute of Microbiology, Czech Academy of Sciences*

**E-mail of the presenting author:** pragyakamal1999@gmail.com

Translational initiation site (TIS) selection is a fundamental and tightly regulated process. It ensures correct reading frame establishment and functional protein production. Errors in TIS recognition lead to non-AUG initiation creating truncated or miscoded proteins. This wastes cellular resources and generates potentially toxic products. While fundamental TIS selection mechanisms are largely understood, precise molecular details of AUG selection remain largely unknown. Specific eukaryotic initiation factors (eIFs) regulate the TIS selection in eukaryotes. These factors facilitate the recruitment of 40S ribosomal subunit, messenger RNA (mRNA), and initiator transfer RNA (tRNA) to form the 48S initiation complex, which then scans the mRNA to locate the start codon. eIF3 is the largest eIF containing 12 subunits and serves as a hub that orchestrates the actions performed by other eIFs. eIF1, alongside eIF2β, helps maintain the 48S complex in an open state during mRNA scanning. Its displacement is a critical step in start codon recognition, as the recognition of Met-tRNA leads to eIF1 dissociation from 40S. eIF1 is known to transiently interact with subunit C of eIF3 in 48S. Our project addresses eIF3c and eIF1 interaction dynamics during start codon recognition. We want to understand the molecular mechanism by which the interaction between eIF1 and eIF3c controls AUG start codon selection and maintains translational initiation fidelity in human cells. We are employing an integrated experimental approach that includes GST-pull down assays, CRISPR-Cas9 genome knock-ins, and Luciferase reporter assays to map the binding interface between eIF1 and eIF3c, generate cell lines with eIF3c variants, and assess AUG recognition efficiency. Ultimately, this research is crucial because abnormal proteins initiated without the standard AUG start codon are linked to various human diseases, such as neurodegenerative disorders like Huntington's disease and cancers like hepatocellular carcinoma. In these cases, altered TIS stringency can drive the overproduction of specific proteins, making their study vital for finding new therapeutic targets and developing effective treatments.

# 17
# Quality control during snRNA biogenesis

**Filip Karásek**[1,2], **Hana Petržílková**[1], **Anton Škríba**[3], **Hana Macíčková Cahová**[3], **David Staněk**[1,2]

1. *Institute of Molecular Genetics of the Czech Academy of Sciences*
2. *Faculty of Science, Charles University*
3. *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences*

**E-mail of the presenting author:** karasekf@img.cas.cz

The biogenesis of new snRNP particles is a strictly regulated process. This project focuses on the biogenesis of the Sm class, which includes U1, U2, U4, and U5. Sm class snRNAs are transcribed by RNA polymerase II and modified at the 5' end by several enzymes during transcription. The first modification is the addition of a 7'-methylguanosine cap to the 5' end. In the next step, 2'-O-methylation is added to the first transcribed nucleotide by the enzyme CMTR1. This modification is recognized by the enzyme PCIF1, which then creates an m6Am modification in the first transcribed nucleotide. This modification is only temporary and is erased by the enzyme FTO demethylase. The 5' end of snRNA is recognized by the nuclear export complex and snRNA is transported through the nuclear pore into the cytoplasm, where the export complex is disassembled. Three essential steps occur during the cytoplasmic phase of biogenesis. The first step, which is well characterized, is the assembly of the Sm-ring. This is an essential step in snRNA biogenesis because if the Sm ring is not assembled, the snRNA is directed to the P-bodies for degradation. The assembly of the Sm ring serves as a signal for TGS1, which hypermethylates the 7'-methylguanosine cap to a 2;2;7-trimethylguanosine cap. During the cytoplasmic phase, the second transcribed nucleotide is 2'-O-methylated by the CMTR2 enzyme. Previous publications have demonstrated for U2 snRNA that the absence of 2'-O-methylation on the second transcribed nucleotide prevents the formation of a functional spliceosome. Presence of the Sm ring and trimethylguanosine cap serves as a bipartite signal for snRNA reimport into the nucleus. In the nucleus, snRNP is targeted to Cajal bodies, where further maturation steps occur. The aim of our project is to describe how the cell distinguishes between wild-type and truncated snRNAs that are capable of assembling an Sm-ring.

Our current data suggest that truncated snRNAs differ in their methylation status at the 5' end and that deviations in methylation may serve as a signal for the cell to exclude such defective snRNAs from biogenesis. We observe that truncated snRNAs interact with innate immunity proteins called IFIT proteins. Overexpression of PCIF1 and CMTR2 leads to a reduction in the interaction of truncated snRNAs with IFIT proteins.

# 18
# Rifampicin as a transcription stimulating agent in Bacillus subtilis

**Petra Sudzinová[1], Tamara Knežová Balgová[1,5], Marek Schwarz[1], Klára Juříková Mikesková[1,5], Karolína Hegrová[1], Dragana Vítovská[1], Priyanka Rawat[1,5], Šárka Bobková[1], Debora Pospíšilová[1,5], Alena Křenková[2], Martin Hubálek[2], Petr Halada[1], Ivan Barvík[3], Tomáš Koval'[4], Jan Dohnálek[4], Jana Wiedermannová[1], Hana Šanderová[1], Libor Krásný[1]**

*1. Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic*
*2. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 160 00 Prague, Czech Republic*
*3. Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, 121 16 Prague 2, Czech Republic*
*4. Institute of Biotechnology of the Czech Academy of Sciences, Průmyslová 595, 252 50 Vestec, Czech Republic*
*5. Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, 128 44 Prague, Czech Republic*

**E-mail of the presenting author:** tamara.balgova@biomed.cas.cz

Rifampicin is a clinically important antibiotic targeting bacterial RNA polymerase (RNAP). It binds to the β subunit of RNAP and stops the synthesis of RNA at the stage of only several nucleotides transcribed. Bacteria have developed various strategies of resistance against this antibiotic (e.g. *rpoB* gene mutation, enzymatic inactivation, active efflux). Recently, it was shown in Actinobacteria that rifampicin induces expression of HelD (Class II), an interaction partner of RNAP, and this contributes to rifampicin resistance. In contrast, no effect of HelD (Class I) on rifampicin resistance was detected in Firmicutes. Here, we re-investigated the possibility of whether *Bacillus subtilis* HelD (phylum Firmicutes) plays a role in rifampicin resistance. Contrary to the published results, we showed that the presence of the *helD* gene increases the minimal inhibitory concentration (MIC) to rifampicin, and its expression was stimulated by rifampicin at the transcriptional level. Next, we identified a region in the 5' untranslated region of the *helD* gene, which is responsible for this induction. Results of experiments addressing the mechanism of regulation of *helD* gene expression and resistance of *B. subtilis* to rifampicin will be presented and discussed.

**Keywords**: HelD, rifampicin, RNA polymerase, *Bacillus subtilis*, antibiotic resistance

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

# 19
# ABCF PROTEIN-MEDIATED RESISTANCE FINE-TUNES TRANSLATOME IN RESPONSE TO ANTIBIOTICS BASED ON THEIR TYPE AND CONCENTRATION

**Markéta Koběrská[1], Ludmila Veselá[1], Michaela Novotná[1], Julie Pokorná[1], Durga Mahor[1], Gabriela Balíková Novotná[1]**

*1. Institute of Microbiology of the CAS; BIOCEV, Vestec, Czech Republic*

**E-mail of the presenting author:** koberska@biomed.cas.cz

Antibiotics play an important role in shaping bacterial communities. Beyond growth inhibition, antibiotics can function as signaling molecules and trigger various cellular responses depending on their target and concentration. A key target for antibiotics is the bacterial ribosome, and ARE ABCF proteins are increasingly recognized as translation factors that rescue antibiotic-stalled ribosomes (1–4). Here, we characterize two closely related ARE ABCF proteins in *Streptomyces coelicolor*, TiaA and Are5sc, and reveal their broader role in antibiotic-responsive regulation. Both proteins fine-tune their own expression, which is controlled by ribosome-mediated transcriptional attenuation in response to lincosamide, streptogramin A, and pleuromutilin (LSAP) antibiotics. Notably, the same resistance activity can either promote or inhibit production of the ABCF protein, depending on the sequence context of the translated mRNA. Comprehensive proteomic analyses further show that ABCF-mediated fine-tuning extends beyond autoregulation to affect unrelated gene networks, including the translation stress-response regulon WblC/WhiB7. These findings highlight ARE ABCFs not only as resistance determinants but also as regulators of antibiotic-responsive translation, adding a new layer of complexity to ribosome-mediated signaling in bacteria.

1. C. R. Fostier et al., FEBS Lett. 595, 675–706 (2021).
2. B. Chen et al., Nat Struct Mol Biol. 21, 152–9 (2014).
3. M. Koberska et al., mBio. 12 (2021), doi:10.1128/mBio.01731-21.
4. F. Ousalem et al., Nat Commun. 15, 1–16 (2024).

# 20
# D-cycloserine resistance in Bacillus subtilis

**Veronika Kočárková[1], Lenka Dejmalová[1], Hana Šanderová[1], Marek Schwarz[1], Tamara Knežová Balgová[1], Jiří Pospíšil[1], Libor Krásný[1]**

*1. Institute of Microbiology of the CAS, Prague, Czech Republic*

**E-mail of the presenting author:** veronika.kocarkova@biomed.cas.cz

The incidence of infections caused by multidrug-resistant bacteria has been increasing in recent decades, while effective therapeutic options remain limited. The broad-spectrum antibiotic D-cycloserine (DCS) is used as a second-line treatment for multidrug- and extensively drug-resistant tuberculosis. Despite its clinical importance, little is known about DCS resistance in bacteria. Here, using *Bacillus subtilis* as a model organism, we identify a novel mechanism of resistance against DCS mediated by YdeK and YdeL proteins. Our preliminary findings suggest that YdeK functions as a DCS exporter while YdeL, a predicted transcription factor, regulates its expression. The current progress of the project, including the regulatory mechanism underlying this resistance, will be presented and discussed.

This work is supported by grant No. 23-06295S from the Czech Science Foundation.

# 21
# All-atom Simulation Model of the Mitochondrial Ribosome

**Rudolf Kvasňovský** [1], **Michal H. Kolář**[1]

*1. University of Chemistry and Technology; Prague, Czech Republic*

**E-mail of the presenting author:** kvasnovr@vscht.cz

Mitochondrial ribosomes (mitoribosomes) are complex biochemical structures composed of several rRNA units and dozens of protein chains. They are located within mitochhondria and are responsible for mitochondrial gene expression during translation, thus fulfilling the same role as other ribosomes. However, their structure differs a lot from that of cytosolic ribosomes. Human mitoribosomes are highly specialised and synthesize only 13 proteins, which all serve as essential subunits of the respiratory chain complexes in the inner mitochondrial membrane.

This project focuses on building and validating an all-atom simulation model of the mitoribosome in an explicit solvent and ion environment. To date, only a few simulation models of mitoribosomes exist, and none of them include the entire mitoribosome in the presence of solvent and ions. The trajectories obtained from these simulations will be used to investigate specific mitoribosomal regions in more detail and to describe their dynamic behaviour.

# 22
# Structural characterisation of senataxin

**Matyas Lanicek**[1,2], **Katerina Sedova**[1], **Marek Sebesta**[1]

1. *CEITEC, Central European Institute of Technology, Masaryk University, Brno, Czechia*
2. *Department of Condensed Matter Physics, Faculty of Science, Masaryk University*

**E-mail of the presenting author:** matyas.lanicek@ceitec.muni.cz

During transcription, RNA polymerases (RNAPs) may pause, increasing the risk of collisions with DNA replication machinery. Such conflicts can lead to replication fork stalling and DNA double-strand breaks. Paused RNAPs may also promote formation of R-loops – structures in which nascent RNA hybridises to its DNA template, exposing the non-template strand as fragile single-stranded DNA, which can cause genomic instability.

Senataxin (SETX), an SF1B-family helicase, differs from its yeast counterpart Sen1 by translocating preferentially on RNA [1]. SETX targets R-loops and functions as a transcription termination factor in a species-specific manner, suggesting the evolution of unique sequence determinants for its activity [1]. These properties position SETX as a key regulator of R-loop metabolism and transcription-replication conflict resolution, critical for genome integrity.

Due to the lack of experimental structural data, we apply structural biology techniques to explore the molecular mechanisms underpinning SETX function. Our poster presents findings which offer initial structural insights into SETX's mode of action.

[1] HASANOVA, Zdenka, Veronika KLAPSTOVA, Odil PORRUA, Richard STEFL and Marek SEBESTA. Human senataxin is a bona fide R-loop resolving enzyme and transcription termination factor. *Nucleic Acids Research* [online]. 2023, 2023-04-11, **51**(6), 2818-2837 [cit. 2025-09-15]. ISSN 0305-1048. Available at: doi:10.1093/nar/gkad092

# 23
# tRNA introns are novel intrinsic antiterminators in plant chloroplasts

**F. Vanessa Loiacono**[1,2]

1. *Charles University (Prague, Czech Republic)*
2. *Max Planck Institute of Molecular Plant Physiology (Potsdam, Germany)*

**E-mail of the presenting author:** vanessa.loiacono@natur.cuni.cz

Transcription termination in plant chloroplasts differs from classical prokaryotic mechanisms. Although it is believed to rely solely on secondary structures folding in the 3' UTRs of genes (an 'intrinsic' type of terminators), plastid terminators lack the typical bacterial motifs of GC-rich hairpins followed by a poly-U tract. Additionally, chloroplast terminators do not efficiently terminate transcription either *in vivo* or *in vitro*.

By screening for potential termination signals in plastid genomes, we noticed that tRNA genes are nearly universally interrupted by introns when co-transcribed with downstream genes. We hypothesized that the presence of the intron prevents premature transcription termination at the highly structured tRNA domains. By removing tRNA introns from the plastid genome, we demonstrate that, in the absence of the intron, RNAP processivity is impaired, resulting in premature termination of RNA synthesis from operons. This, in turn, leads to mutant phenotypes due to low accumulation of the gene product(s) from the downstream cistron(s).

We propose that introns have evolved in chloroplast genomes (i) to adapt to modern RNAPs, which are more sensitive to structured sequences than their prokaryotic counterparts, and (ii) to facilitate the incorporation of tRNA genes into operons, thus increasing gene density and providing additional opportunities for co-regulation.

Finally, I will discuss the current efforts towards the design of short, efficient termination signals for synthetic and biotechnological applications.

# 24
# DECIPHERING THE SIGNALING FUNCTION OF ANTIBIOTIC RESPONSIVE ABC-F ATPase LmrC

**Durga Mahor**[1], **MARKÉTA KOBĚRSKÁ**[1], **MUFARRAH MEHBOOB**[1], **Jakub Musil**[1], **GABRIEL Demo**[1], **ZDENĚK KAMENÍK**[1], **GABRIELA BALÍKOVÁ NOVOTNÁ**[1]

*1. Institute of Microbiology of the CAS*

**E-mail of the presenting author:** durga.mahor@biomed.cas.cz

ARE-ABCF proteins provide antibiotic resistance by displacing antibiotics from their binding sites on the 50S ribosomal subunit. However, our work revealed an additional regulatory function for a specific ARE-ABCF ATPase, **LmrC**, found in the lincomycin-producing bacteria *Streptomyces lincolnensis*. LmrC serves a dual purpose: it provides low-level antibiotic resistance while also acting as a **signal transducer**. Upon sensing lincosamide antibiotics, LmrC triggers a signaling cascade that activates the transcription of the regulatory gene ***lmbU***, thereby facilitating lincomycin production.[1]

To investigate the mechanism by which LmrC regulates the transcription of *lmbU*, we employed a combination of genetic, biochemical, and structural analyses. Our findings indicate that the 5' untranslated region (5' UTR) of *lmbU* contains a small regulatory open reading frame (uORF) that is translated in vitro and is essential for the transcriptional activation of *lmbU*. Disruption of this uORF leads to the failure of activation in the *lmbU* transcriptional reporter. Additionally, with 3' RACE, we identified a premature transcriptional terminator that overlaps with the internal promoter within the *lmbU* 5' UTR. Collectively, these results suggest that the transcription of *lmbU* is regulated by ribosome-mediated attenuation. In this model, ribosome stalling at the uORF initiates a structural rearrangement of the 5' UTR, resulting in the formation of a non-permissive, anti-terminator conformation.

Furthermore, we observed that elevated expression levels of LmrC alone are sufficient to activate *lmbU* transcription. We propose that high levels of LmrC induce ribosome stalling, which elucidates the mechanistic basis of LmrC-mediated regulation. In vivo reporter assays and single-particle cryo-electron microscopy analyses of the ribosomal complex involving LmrC and the lmbU 5' UTR are currently underway.

Reference

1. M. Koberska, L. Vesela, V. Vimberg, J. Lenart, J. Vesela, Z. Kamenik, J. Janata, G. Balikova Novotna, and G. B. Novotna, *MBio*, 2021, **12**, 2020.10.16.343517.

Acknowledgement

This work was supported by the European Union, project „MSCA Fellowships CZ No. 2 (Institute of Microbiology of the CAS, v. v. i.)”, reg. number CZ.02.01.01/00/22_010/0006118

# 25
# Tackling the mechanism of RNA uridylation in viral infection

**Ayat Masri**[1], **Anna Vlčková**[1], **Lucie Nepovímová**[1], **Liya Mukhamedova**[1], **Pavel Plevka**[1], **Stepanka Vanacova**[1]

*1. CEITEC, Masaryk University, Kamenice 5/E35, Brno, Czech Republic*

**E-mail of the presenting author:** 253201@muni.cz

Post-transcriptional RNA modifications are a significant aspect of gene expression control. Among these, 3' RNA uridylation, which is catalyzed by terminal uridylyl transferases (TUT4/7), controls RNA stability, processing, and degradation. While uridylation has been linked to developmental processes and cancer, its involvement in host-pathogen interactions is under explored. Recent research suggests uridylation acts as an antiviral strategy by destabilizing viral RNAs and thus limiting viral replication (Gupta et al., 2023). Here, we aim to address the potential of TUTases in infection with Echovirus 18 (E18), a clinically relevant enterovirus associated with meningitis. The research is structured around two aims: (1) to identify cofactors and regulators of TUT4/7 in human cells using CRISPR-based tagging, proximity-based interactome mapping; and (2) to define the impact of uridylation on viral replication and host–virus RNA networks during E18 infection. Preliminary results show successful generation of HEK293T cell lines with endogenous tagging of DIS3L2, TUT4, and TUT7, enabling proximity-based interactome mapping. Current analyses are validating potential regulators and optimizing viral infection models.

Reference:

Gupta A, Li Y, Chen SH, Papas BN, Martin NP, Morgan M. TUT4/7-mediated uridylation of a coronavirus subgenomic RNAs delays viral replication. Commun Biol. 2023 Apr 21;6(1):438. doi: 10.1038/s42003-023-04814-1. PMID: 37085578; PMCID: PMC10119532.

# 26
# safRa: Splicing Annotation for Aberrant Fraction Analysis in Hereditary Cancer Diagnostics

**Kateřina Matějková**[1], **Petra Kleiblová**[1], **Zdeněk Kleibl**[1], **Petr Nehasil**[1]

*1. Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University and General University Hospital in Prague*

**E-mail of the presenting author:** katerina.matejkova@lf1.cuni.cz

Accurate characterization of splice junctions is critical for understanding the role of alternative and aberrant splicing in hereditary cancers and other genetic disorders. We present **safRa**, an R-based computational tool that annotates splice junctions and quantifies their relative abundance compared to corresponding wild-type events.

Built using the syntax and logic of the **tidyomics** ecosystem, **safRa** provides a streamlined interface compatible with the **tidyverse** and the **Bioconductor** framework, supporting readable code and seamless integration into bioinformatics workflows. The tool accepts splice junction coordinates and RNA-seq coverage data and supports custom transcript annotations, offering flexibility beyond default references such as **MANE Select**.

A core feature of **safRa** is its ability to identify wild-type counterparts for alternative junctions and compute their **relative usage**, offering biologically anchored quantification. It further **annotates** each junction with genomic context, coding potential, and exon involvement.

Although similar tools are available, they often demand advanced bioinformatics expertise or custom scripting and may not incorporate the latest developments in splicing interpretation. In contrast, **safRa's** implementation as a **Bioconductor** package offers an accessible, up-to-date solution that lowers the barrier for researchers and clinicians without extensive programming experience.

Aligned with **ACMG/AMP recommendations** from the **ClinGen SVI Splicing Subgroup**, **safRa** supports evidence-based variant interpretation. By delivering detailed and accessible splicing analysis, it facilitates high-resolution RNA-seq interpretation in both research and diagnostic settings.

# 27
# Crystallization of protein-DNA complexes of virulence-associated transcriptional regulators from Staphylococcus aureus

**Anna Maďarová[1,2], Jana Škerlová[1], Adéla Fejfarová[1,2], Tereza Vučková[1], Irena Sieglová[1], Milan Fábry[1], Markéta Šoltysová[1], Pavlína Řezáčová[1]**

*1. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo n. 2, 16000 Prague, Czech Republic*
*2. Charles University, Faculty of Science, Department of Cell Biology, Viničná 7, Prague 2, Czech republic*

**E-mail of the presenting author:** anna.madarova@uochb.cas.cz

Antibiotic resistance is deemed as one of the greatest threats of the 21st century. One of the leading pathogens responsible for this crisis is multi-drug resistant *Staphylococcus aureus*, whose virulence is intricately regulated by quorum sensing-associated transcriptional regulators (1). Given their crucial role in the expression of virulence factors and species-specificity, transcriptional regulators of the Sar family represent particularly attractive targets for a completely novel class of antibiotic compounds (2). Yet in order to successfully target these regulators, a profound knowledge about their molecular mechanism of action is required. In many cases, 3D protein-DNA complexes are the missing pieces of information needed for unraveling the mode of action of these regulators as well as for uncovering possible sites for their inhibition.

In the herein presented work, we set out to fill these gaps by setting up the crystallization of selected virulence-associated transcriptional regulators in complex with their native operators. We successfully purified and characterized the SarR and SarA regulators, tested their ability to bind to designed duplexes derived from native recognition sites and tested various crystallization conditions. After obtaining initial crystals, we are intensively working on the optimization of protein constructs, DNA duplexes as well as crystallization techniques and conditions.

References:

1. Cheung, A. L., Koomey, J. M., Butler, C. A., Projan, S. J., & Fischetti, V. A. (1992). Regulation of exoprotein expression in Staphylococcus aureus by a locus (sar) distinct from agr. Proceedings of the National Academy of Sciences, 89(14), 6462–6466. https://doi.org/10.1073/pnas.89.14.6462

2. Balamurugan, P., Praveen Krishna, V., Bharath, D., Lavanya, R., Vairaprakash, P., & Adline Princy, S. (2017). Staphylococcus aureus Quorum Regulator SarA Targeted Compound, 2-[(Methylamino)methyl]phenol Inhibits Biofilm and Down-Regulates Virulence Genes. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.01290

This work is supported by the Project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, co-funded by the European Union.

# 28
# EXPLORING THE ANTIBIOTIC-RESPONSIVE REGULATORY FUNCTION OF ABCF PROTEINS IN ANTIBIOTIC-PRODUCING BACTERIA

**Mufarrah Mehboob**[1], **MARKÉTA KOBĚRSKÁ**[1], **ANINDA MAZUMDAR**[1], **ZDENĚK KAMENÍK**[1], **GABRIELA BALÍKOVÁ NOVOTNÁ**[1]

*1. Institute of Microbiology AS CR; Biocev, Průmyslová 595, 252 50 Vestec, Česká Republika*

**E-mail of the presenting author:** mufarrah.mehboob@biomed.cas.cz

ABCF proteins are cytosolic ATPases of the ABC superfamily, which includes proteins involved in the regulation of translation or the protection of the ribosome against antibiotics. Despite their different biological functions, all characterized ABCFs bind to the same site on the ribosome, and their common feature is ATP-dependent modulation of the peptidyl transferase center. However, the vast majority of the 30 bacterial ABCF subfamilies have not yet been characterized. Our recent findings have shown that one of the ABCFs, LmrC, activates lincomycin biosynthesis in response to lincosamide antibiotic added to the medium [1]. However, it is not known how widespread ABCF-mediated antibiotic signaling is.

Bioinformatic search revealed ABCF proteins encoded within BGCs for some ribosome-binding antibiotics. Furthermore, the type of ABCF protein encoded in a particular BGC correlates with the mode of antibiotic binding to the ribosome. We hypothesize that these ABCF proteins participate in signaling cascades that initiate antibiotic production in response to the presence of antibiotics similar to LmrC. For the study, we have already selected candidate strains containing representatives of the ARE4, AAF1 and AAF2 subfamilies of ABCF proteins. We will try to find out which proteins are directly regulated by ABCF proteins and to characterize ABCF signaling in heterologous host. The study of these biosynthetic pathways at the genetic level may in the future allow the manipulation of genes to engineer new strains capable of producing novel antibiotics or increasing the yield of existing antibiotics.

REFERENCES: [1] Koberska, M., Vesela, L., Vimberg, V., Lenart, J., Vesela, J., Kamenik, Z., ... & Balikova Novotna, G. (2021). Mbio, 12(5), e01731-21.

# 29
# GlcR regulation of ptsG expression is crucial for resistance to sublancin

**Sára Michková**[1], **Dragana Vítovská**[1], **Jiří Pospíšil**[1], **Libor Krásný**[1]

*1. Laboratory of Microbial Genetics and Gene Expression, Institute of Microbiology of the Czech Academy of Sciences, 142 20, Prague 4, Czech Republic*

**E-mail of the presenting author:** sara@michek.com

The antimicrobial peptide (AMP) sublancin is a 37 amino acid long glycopeptide synthesized by the Gram-positive soil bacterium *Bacillus subtilis*. Previous research on sublancin has revealed its antibiotic potential as it is lethal for various Gram-positive bacteria, including pathogens, such as *Streptococci* or *Staphylococci*. As with other antibioitics, resistance to sublancin can arise. Despite decades of research, the determinants of sublancin resistance are still poorly understood.

To reveal these determinants, we performed whole-genome sequencing of sublancin-resistant *B. subtilis* mutants. This screen identified mutations in two genes - *ptsG* or *glcR,* encoding PtsG and GlcR, respectively. PtsG is a membrane permease of the phosphotransferase system (PTS), responsible for the transport of glucose into the cell. GlcR is a transcriptional factor that functions as a repressor of the *glcR-phoC* operon. Consistent with the screen, deletion of either gene rendered the bacterium resistant to sublancin.

The role of PtsG and GlcR was further investigated using RT-qPCR that showed that *ptsG* expression is downregulated by an order of magnitude in the *glcR* mutant strains as well as in a *ΔglcR* strain, suggesting a regulatory interplay between these two proteins. Subsequent *in vitro* transcriptions with purified recombinant GlcR allowed us to shed light on this regulatory interplay.

Finally, hypotheses about the mechanism of how mutations in these two proteins provide the cell with sublancin resistance will be presented and discussed.

# 30
# RNA tailing is a regulatory mechanism of Euglena photosynthesis

**Jayaram Moorthy**[1], **Jiří Pergner**[2], **Vojtech Žárský**[2], **Anna Hrčková**[1], **Karolína Kmeťová**[1], **Magdaléna Hoňkova**[2], **Ragul Ravikumar**[2], **Milan Marcincak**[1], **Elizabeth M. Štefl**[1], **Vojtěch Jakubík**[2], **Dietmar Riedel**[3], **Marek Eliáš**[2], **Štěpánka Vaňáčová1**[1]

*1. 1.Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5/E35, 625 00 Brno, Czech Republic*
*2. 2.Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00, Ostrava, Czech Republic*
*3. 3.Facility for Electron Microscopy, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, 37077 Göttingen, Germany*

**E-mail of the presenting author:** 544697@muni.cz

RNA tailing, the non-templated addition of nucleotides to the 3' termini of RNAs, is known to influence transcript stability, expression, and turnover through the activity of terminal nucleotidyl transferases (TRFs) (1).While this process has been studied in the nucleus and cytoplasm, its role in organelles remains largely unexplored . To investigate RNA tailing in plastids, we examined plastids of E. *gracilis* and identified two plastid-localized TRF proteins, designated pTNT1 and pTNT2 (2).CRISPR/Cas9-mediated knockout of both genes produced mutant strains with yellowish pigmentation, reduced chlorophyll content, and disorganized plastids, as revealed by transmission electron microscopy. Proteomic profiling of the mutants demonstrated broad downregulation of plastid-localized proteins, particularly core subunits of photosystems I and II, the cytochrome b6f complex, and ATP synthase. Transcriptome analyses further showed that loss of pTNT1 or pTNT2 selectively impaired splicing of distinct subsets of group II introns, including those within *psbB* and *psbD*, which encode essential photosynthetic proteins. RT-PCR confirmed intron retention in these transcripts, linking defective RNA processing to reduced protein accumulation. These results suggest that intron retention in plastidial pre-mRNAs disrupts photosynthetic complex biogenesis, which in turn destabilizes the broader photosynthetic machinery. Our findings uncover an unexpected role for plastid-localized TRFs in group II intron splicing, establishing RNA tailing as a critical factor in chloroplast gene expression and plastid function in *E.gracilis*.

Reference

1.Chen J, Li X, Dong X, Wang X. Functions and mechanisms of RNA tailing by nucleotidyl transferase proteins in plants. Front Plant Sci. 2024 Oct 15;15:1452347. doi: 10.3389/fpls.2024.1452347. PMID: 39474218; PMCID: PMC11518768.

2.Novák Vanclová AM, Zoltner M, Kelly S, Soukal P, Záhonová K, Füssy Z, Ebenezer TE, Lacová Dobáková E, Eliáš M, Lukeš J, Field MC. Metabolic quirks and the colourful history of the Euglena gracilis secondary plastid. New Phytologist. 2020 Feb;225(4):1578-92.

# 31
# Fidaxomicin resistance conferring RNA Polymerase mutations exhibits collateral sensitivity to Rifampicin

**Saran Natarajan**[1], **Marek Schwarz**[1], **Libor Krásný**[1]

*1. Institute of Microbiology of the Czech Academy of Sciences*

**E-mail of the presenting author:** saran.natarajan@biomed.cas.cz

Fidaxomicin (FDX) is a narrow spectrum macrocyclic antibiotic produced by the fermentation of tiacumicin metabolites by the actinomycete *Dactylosporangium aurantiacum*. It is currently recommended as the first-line treatment option for *Clostridioides difficile* infections. FDX acts by targeting RNA polymerase (RNAP), inhibiting the formation of the open complex, an essential intermediate in the transcription initiation pathway. Mutations in the FDX binding site on RNAP contributes to FDX resistance. However, the collateral responses of these mutations on other antibiotics are unknown. We aimed to study collateral responses of FDX resistance associated RNAP mutations to other antibiotics using the gram-positive model organism, *Bacillus subtilis*. We generated FDX resistant *B. subtilis* mutants by exposing them to concentrations of FDX between 4 to 8 µg/mL. For selected clones, minimum inhibitory concentrations (MIC) of FDX were 16-32fold higher than for the wild-type parental strain. MIC testing against Rifampicin revealed few strains exhibiting increased sensitivity to Rifampicin. Whole genome sequencing revealed novel mutations near the FDX binding site in *rpoB* gene, albeit at a low frequency. The results will be presented and discussed.

This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

# 32
# Structural study of Ap4A-RNA biosynthesis by mammalian RNA polymerase II

**Ondřej Nešuta**[1], **Jan Petrovský**[2], **Marek Šebesta**[2], **Richard Štefl**[2], **Hana Cahová**[1]

*1. Institute of Organic Chemistry and Biochemistry of the CAS, Flemingovo náměstí 2, Prague 6, Czechia*
*2. CEITEC-Central European Institute of Technology, Masaryk University, Brno CZ-62500, Czechia*

**E-mail of the presenting author:** ondrej.nesuta@uochb.cas.cz

RNA polymerase II is a key eukaryotic enzyme complex responsible for the synthesis of DNA-encoded precursors of protein-coding transcripts, as well as long non-coding RNAs, small nucleolar RNAs, and microRNAs. Most RNA polymerase II transcripts are initially synthesized as 5'-triphosphate (ppp-) RNAs. Once a certain transcript length is reached, the capping enzyme is recruited to RNA polymerase II, converting the 5'-triphosphate end into a guanosine triphosphate. This step represents the beginning of the canonical co-transcriptional RNA capping process that ultimately produces the 7-methylguanosine RNA cap (cap 0).

Recent discoveries of non-canonical RNA caps—including nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), and dinucleoside polyphosphates—have raised questions about their mechanisms of formation. Here, we investigate the hypothesis that RNA polymerase II can directly use free diadenosine tetraphosphate (Ap4A), which is abundant in the nucleus, as a non-canonical initiating nucleotide to generate Ap4A-capped RNA during transcription.

Our biochemical assays demonstrate that porcine RNA polymerase II efficiently produces Ap4A-RNA in vitro, in quantities even greater than canonical ppp-RNA. To gain structural insight into this process, we employed cryogenic electron microscopy (cryo-EM) of RNA polymerase II bound to a DNA template and Ap4A-RNA product. Finally, we show that Ap4A-RNA is not a substrate for the canonical capping enzyme, suggesting that Ap4A caps represent a distinct, stable, non-canonical RNA modification.

We also believe that Ap4A-RNA biogenesis may represent an alternative RNA regulatory pathway, and that understanding this process could provide important information about its biological role.

# 33
# Uniqueness over universality: LSAP antibiotics are sequence-dependent translation inhibitors

**Michaela Novotná[1,2], Thibaud T. Renault[3], Mélanie Gillard[3], Vojtěch Kovařovic[1], Julie Pokorná[1], Markéta Koběrská[1], C. Axel Innis[3], Gabriela Balíková Novotná[1]**

*1. Institute of Microbiology, Czech Academy of Sciences, BIOCEV, Vestec, 25250, Czech Republic*
*2. Faculty of Science, Department of Genetics and Microbiology, Charles University in Prague, Prague, 12800, Czech Republic*
*3. Acides Nucléiques: Régulations Naturelle et Artificielle, Université de Bordeaux, Institut National de la Santé et de la Recherche Médicale U1212, Centre National de la Recherche Scientifique UMR5320, Bordeaux Biologie Santé, Bordeaux, 33000, France*

**E-mail of the presenting author:** michaela.novotna@biomed.cas.cz

Lincosamide, streptogramin A, and pleuromutilin (LSAP) antibiotics inhibit bacterial translation by binding to the catalytic centre of the ribosome. However, despite their extensive use in medicine and research as inhibitors of translation initiation, their mode of action has never been studied in detail. Here, we applied ribosome profiling using both the standard Ribo-seq and the recently developed iTP-seq (1) approach to identify peptide-encoding transcripts that promote ribosome stalling in the presence of LSAP antibiotics. We show that these drugs are not universal inhibitors of ribosome function, as commonly assumed, but instead display distinct, sequence-dependent modes of action that are dictated by the amino acid sequence of the nascent peptide. This was independently validated by *in vitro* and *in vivo* fluorescence reporter assays. Bacteria exploit this sequence-dependent inhibition to discriminate between antibiotics and to regulate expression of resistance genes *via* ribosome-mediated attenuation at the mRNA level. Building on these findings, we are now validating how LSAP antibiotics modulate resistance gene regulation in native systems. Elucidating the mode of action of LSAP antibiotics provides a conceptual framework for the rational development of next-generation antibiotics that can overcome resistance.

(1) Seip, B. et al. (2018). Ribosomal stalling landscapes revealed by high-throughput inverse toeprinting of mRNA libraries. Life Sci Alliance *1*.

# 34
# Ambiguous translation termination in Blastocrithidia nonstop

**Puranjaya Pancholi**[1,2], **Nathalia Ballesteros** [1,2], **Julie Kovářová**[1], **Jiri Koubek**[1], **Karel Harant**[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. *Laboratoř hmotnostní spektrometrie, Přírodovědecká fakulta, Univerzeta Karlova*

**E-mail of the presenting author:** puranjaya.pancholi@paru.cas.cz

The universality of the genetic code is challenged by organisms that reassign stop codons. In the parasitic protist *Blastocrithidia nonstop*, a close relative of the human pathogens *Trypanosoma* and *Leishmania*, all three stop codons are unevenly distributed within ORFs across the genome and have been reassigned as sense codons. Decoding of this noncanonical genetic code is facilitated by two cognate tRNAs that decode the stop codons UAG and UAA as glutamate. For the UAG stop codon, *B. nonstop* uses a near cognate tRNATrpCCA bearing a noncanonical 4-base pair (bp) long anticodon stem (AS). Notably, UAA retains its function as a termination codon, indicating dual functionality. In addition, UAA codons are highly enriched in 3' UTRs, raising questions about translation termination fidelity. To investigate this phenomenon, we performed mass spectrometry on *B. nonstop* and identified over 25,000 peptides, including 300 C-terminal peptides. Using an *in silico* extended coding sequence database, we discovered that translation mostly terminates at the annotated stop codon. However, in a substantial number of cases, translation continues, producing extended proteins. Our results suggest that termination in *B. nonstop* via single UAA stop codon may be insufficient and controlled by context-dependent, controlled by an alternative mechanism.

# 35
# NUDT2 and Non-Canonical RNA Decapping: Insights into Disease-Specific Regulation of NudiX Enzymes

**Gibrán Pedraza-Vázquez**[1], **Hana Cahová**[1]

*1. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences*

**E-mail of the presenting author:** gibran.vazquez@uochb.cas.cz

The nucleoside diphosphates linked to moiety-X (NudiX) hydrolase family comprises twenty-four human enzymes involved in hydrolyzing nucleoside diphosphates linked to other moieties, with essential roles in nucleotide metabolism, signaling, and genome integrity. While canonical decapping by DCP2 (NUDT20) has been extensively characterized, the contribution of other NudiX enzymes to *non-canonical* RNA decapping remains less understood. Recent discoveries of non-canonical RNA caps (NAD, $Ap_2N$, and $Ap_4A$), suggest that their regulated removal may influence RNA stability, stress response, and cellular adaptation.

Among NudiX members, NUDT2 remains poorly characterized in human disease contexts. Unpublished biochemical data from our group indicate that the preferred substrate of NUDT2 is $Ap_4A$-RNA rather than free $Ap_4A$, suggesting a direct role in non-canonical RNA cap metabolism. To investigate disease relevance, we performed a large-scale transcriptomic survey of NudiX expression using the *recount3* framework, encompassing 72 datasets and 26,333 RNA-seq samples from TCGA, GTEx, and SRA. Our analysis revealed disease-specific regulation of NUDT2: it is significantly upregulated in hepatocellular carcinoma and cholangiocarcinoma, yet downregulated in metabolic liver disorders such as NAFLD and NASH. These contrasting patterns point to context-dependent roles, whereby NUDT2 may facilitate RNA turnover and stress adaptation in cancer, but its reduced expression in chronic metabolic disease could impair adaptive RNA metabolism.

Moreover, evidence from the Human Protein Atlas highlights a striking discordance between NUDT2 transcript and protein abundance, suggesting post-transcriptional regulation, potentially via miRNAs. Such multilayered control may be essential to fine-tune RNA decapping pathways and prevent inappropriate RNA degradation.

Together, our findings establish NUDT2 as a key player in non-canonical RNA cap metabolism with potential roles in pathophysiology. By integrating biochemical and transcriptomic analyses, this work opens new perspectives on the regulatory significance of $Ap_4A$-RNA and highlights NUDT2 as a promising target for future therapeutic strategies.

# 36
# From DNA to RNA: Parallel Sequencing Uncovers Splicing-Impacting Variants in MMR Genes

**Tatana Ptackova**[1], **Marta Cerna**[1], **Katerina Matejkova**[1,3], **Petra Kleiblova**[1,2]

*1. Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic*
*2. Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic*
*3. Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, Czech Republic*

**E-mail of the presenting author:** tatana.dolezalova@lf1.cuni.cz

Next-generation sequencing (NGS) technology has become the analytical standard for germline DNA testing and the diagnosis of hereditary diseases, including cancer. The growing use of this technology requires accurate sample identification, independent confirmation of prioritized variants, and their functional and clinical interpretation. To streamline these processes, we earlier introduced paired DNA and RNA capture-based NGS using the CZECANCA capture panel, which is routinely used for DNA analysis of hereditary cancer predisposition.

We analyzed 4500 patient samples collected over the past decade using parallel DNA/RNA NGS. This strategy enabled us to assess the impact of each variant on pre-mRNA splicing directly in patient-derived RNA. By coupling these findings with splicing prediction tools that incorporate deep learning and artificial intelligence, we were able to systematically prioritize variants and classify them according to ACMG guidelines.

For this study, we present data from genetic testing of 114 Czech patients who were clinically suspected of having Lynch syndrome and were having splicing affecting variant. We performed detailed comparision highlighting discrepancies, concordances, and key findings that underscore the importance of integrating multiple tools in variant interpretation. We identified 3 novel pathogenic variants causing splicing aberrations that would have remained undetected or uncertain by DNA-based testing alone.

This combined DNA/RNA strategy accelerates the reclassification of variants of uncertain significance (VUS) and also enhances the clinical relevance of germline testing by linking genetic changes to their functional consequences. We anticipate that its broader application will improve diagnostic yield, reduce unnecessary follow-up testing, and support more precise genetic counseling and patient management. This work was supported by the Ministry of Health of the Czech Republic (grant projects NU23-03-00150 and RVO-VFN 64165) and Charles University (SVV 260631).

# 37
# The role of coilin in snRNP maturation

**Nenad Radivojević**[1,2], **Veronika Holotová**[1], **Martina Groušlová**[1], **David Stanek**[1]

1. *Institute of Molecular Genetics of the Czech Academy of Science, Prague*
2. *Department of Genetics and Microbiology, Charles University, Prague*

**E-mail of the presenting author:** nenad.radivojevic@img.cas.cz

Cajal bodies (CBs) are membraneless nuclear organelles involved in the metabolism of spliceosomal small nuclear ribonucleoproteins (snRNPs). During their biogenesis, snRNPs have been observed to transiently localize to CBs, whereas disruption of their maturation leads to their accumulation. While the importance of CBs in snRNP maturation is clear, the precise mechanism and function remain elusive. Coilin, a CB marker protein that has been shown to provide scaffolding and induce CB formation, may serve as a targeting signal and quality control factor in the final steps of snRNP biogenesis. Previous studies have shown that coilin's Tudor-like and RG-box domains interact with Sm proteins and the SMN complex, respectively, while iCLIP experiments indicate its association with different classes of RNA. In this study, we aim to elucidate the nature of the interaction between coilin and snRNPs and determine whether coilin can discriminate between mature and immature snRNP particles. Here, we identify the RG box as an RNA-binding domain capable of pulling down snRNA in vitro. In particular, arginines within the RG-box provide the interaction interface through electrostatic interactions. In addition, we identified two loops within the Tudor-like domain that are important for interaction with the Sm ring. Using the C214 coilin fragment and several of its mutant variants, we have shown that both the RG box and the Tudor-like domain are required for efficient association with U2 snRNP. Furthermore, we show that C214 coilin fragment preferentially interacts with immature snRNPs, and propose the quality control mechanism which regulates the final step of snRNP maturation.

# 38
# Beyond RNA Polymerase Mutations: The interplay of Methyltransferases in Rifampicin Resistance

**Priyanka Rawat**[1], **Šárka Bobková**[1], **Marek Schwarz**[1], **Kristína Spustová**[2], **Anton Škríba**[2], **Hana Cahová**[2], **Libor Krásný**[1], **Jana Wiedermannová**[1]

*1. Institute of Microbiology of the Czech Academy of Sciences, Prague, Czechia*
*2. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czechia*

**E-mail of the presenting author:** priyanka.rawat@biomed.cas.cz

Antibiotic resistance is a growing global concern, necessitating novel strategies of action against it. Understanding the resistance mechanisms available to bacteria is crucial for developing such strategies. To reveal the existing mechanisms of bacterial response to the antibiotic rifampicin, *Bacillus subtilis* was treated with a sub-inhibitory concentration of the antibiotic, followed by proteomics analysis. Upregulation of several proteins was detected, including proteins known to be involved in rifampicin resistance, validating the approach. Proteins previously unsuspected to play roles in this process were also identified, such as uncharacterized putative methyltransferases (MTases). With minimal existing research on MTases in the context of rifampicin resistance, they present a unique opportunity for novel discoveries. They could potentially modify DNA, RNA, proteins, or antibiotics by attaching a methyl group and therefore affecting the gene expression, or the localization, stability, activity or structure of the target molecules.

Our research focuses on a putative rRNA MTase that is overexpressed during rifampicin stress. Its gene is evolutionarily conserved and positioned upstream of the rifampicin resistance mutational hotspot, supporting its role in antibiotic resistance.

Phenotypic analysis of the MTase deletion mutant confirmed the impaired growth under rifampicin treatment. Additionally, co-immunoprecipitation assays identified ribosomal proteins as interacting partners of the FLAG-tagged MTase. To pinpoint the methylation target site and assess changes in methylation in response to rifampicin, we performed direct RNA sequencing. Analysis of the data will provide insights into the role of the MTase in rifampicin stress. Understanding the mechanisms will help us develop new strategies to combat rifampicin resistance.

# 39
# The role of ALKBH5 molecular interactions in mRNA processing

**Praveenkumar Rengaraj**[1,2], **Aleš Obrdlík**[1], **Shwetha Krishna**[1], **Michaela Dohnalkova**[1], **Štěpánka Vaňáčová**[1]

1. *Central European Institute of Technology, Brno, Czech Republic*
2. *National Centre for Biomolecular Research, Faculty of Science, Brno, Czech Republic*

**E-mail of the presenting author:** praveenkumar.rengaraj@ceitec.muni.cz

ALKBH5 is an m6A mRNA demethylase found across different species of life. At the cellular level, the removal of m6A by ALKBH5 is believed to affect the mRNA processing, export, localization, stability, and translation. ALKBH5 is essential for mouse germ line development and its altered expression has been linked to cancer progression in humans. However, ALKBH5 depletion has only minor effect on the overall m6A levels in nascent and steady state mRNAs. Despite multiple studies on ALKBH5, we are still lacking good understanding of its molecular function. Our recent interactome analyses revealed that ALKBH5 interacts with several mRNA processing and export factors. To further tackle the mechanism of ALKBH5 in these events, we performed transcriptomics analysis of ALKBH5 knock-out (KO) HEK293T cells. We observed dramatic expression changes in RNA pol-II transcripts accompanied by splicing alterations. To gain deeper insights into these splicing defects, we analysed publicly available datasets, where we observed exon skipping occurring more frequently than other splicing events. Furthermore, our results from high-throughput CRISPR/Cas9 genetic screening show potential genetic interactions between ALKBH5 and factors involved in splicing and mRNA export, corroborating our proteomic and transcriptomic data. Altogether, our results reveal the importance of ALKBH5 in mRNA processing and export.

# 40
# Characterization of transcription factors regulated by CDK12

**Anna Rožková**[1], **Peter Maník**[1], **Kateřina Hanáková**[1], **Zbyněk Zdráhal**[1], **Dalibor Blažek**[1]

*1. Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czechia*

**E-mail of the presenting author:** 521981@mail.muni.cz

Elongation is a phase of the transcription cycle during which the synthesized nascent RNA is extended and the transcription complex moves along the gene body. The elongation complex (EC) associates with RNA polymerase II and is composed of many proteins that are essential for efficient gene transcription and prevent premature termination. The activities of many of these factors depend on phosphorylation by cyclin-dependent kinases (CDKs). Transcriptional CDKs include CDK12, whose substrates have not yet been identified. The aim of the project is to characterize a transcription factor that is part of the EC and is a potential substrate of CDK12.

# 41
# Enzymatic Synthesis of Base-Modified XNA Using Engineered DNA Polymerases

**Samanta Rožánková**[1,2], **Michal Hocek**[1,2]

1. *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences*
2. *Department of Organic Chemistry, Faculty of Science, Charles University in Prague*

**E-mail of the presenting author:** samanta.rozankova@uochb.cas.cz

Nucleic acids find many applications in the fields of biotechnology and medicine, but in their natural form, they suffer from inherent narrow chemical diversity and susceptibility to degradation by nucleases. The efforts aimed at overcoming these drawbacks center around the alteration of key structural elements of nucleic acids, with the resulting xenobiotic nucleic acids (XNAs) serving as promising alternatives to the canonical genetic polymers. Modifications at the 2'-position of ribose were determined to be crucial in minimizing nuclease cleavage, and by altering properties of the XNA also improving duplex stability [1], thus becoming an essential component of therapeutic oligonucleotides (ONs). The use of 2'-modifications is common practice in most existing therapeutic ONs; however, there is only a handful of works exploring enzymatic synthesis of dually sugar- and nucleobase-modified XNAs. In this work, we focus on the enzymatic construction of nucleobase-modified 2'F XNA, utilizing three primer-dependent engineered thermophilic DNA polymerases (SFM4-3 [2], 2M [3], TGK [4]) and methods previously developed for the synthesis of nucleobase-modified RNA [5]. A set of four base-modified **2'F NRTPs** was synthesized, each bearing a modification on the nucleobase of distinct chemical nature, and tested in primer extension experiment (PEX) in combination with natural NTPs, as well as with 2'F NTPs bearing natural nucleobases. The resistance of the RNA, as well as the 2'F XNA segment of the synthesized oligonucleotide to digestion by DNase allowed easy removal of the DNA template by treatment with DNase and therefore a convenient route to purification and subsequent characterization of the oligonucleotide product. Primer extension reactions employing combination of two and three **2'F NRTPs** paved the way to the synthesis of hypermodified 2'F XNA of various lengths.

This work was funded by the Ministry of Education, Youth and Sports of the Czech Republic grant: RNA for therapy (CZ.02.01.01/00/22_008/0004575).

References:

[1] Zhu, G. *et al.* Front. Bioeng. Biotechnol. 2021, *9*, 792489.

[2] Chen, T. *et al.* Nat. Chem. 2016, *8*, 556–562.

[3] Freund, N. *et al.* Nat. Chem. 2023, *15*, 91–100.

[4] Cozens, C. *et al.* Proc. Natl. Acad. Sci. USA 2012, *109*, 8067–8072.

[5] Brunderová, M. *et al.* Nat. Commun. 2024, *15*, 3054.

# 42
# The role of FTO and m6Am in snRNA processing

**Veronika Rájecká[1], Květoslava Brožinová[1], Shwetha Krishna[1], Aleš Obrdlík[1], Mary Anne O'Connell[1], Štěpánka Vaňáčová[1]**

*1. CEITEC, Masaryk University, Brno, Czech Republic*

**E-mail of the presenting author:** veronika.rajecka@ceitec.muni.cz

Pre-mRNA splicing is a highly complex and tightly regulated process in eukaryotic cells, involving multiple layers of control for both constitutive and alternative splicing. Small nuclear RNAs (snRNAs) transcribed by RNA polymerase II possess an N6,2'-O-dimethyladenosine ($m^6Am$) modification at the first transcribed nucleotide adjacent to the cap structure. This modification can be demethylated by the adenosine demethylase FTO, resulting in alterations in exon splicing patterns. FTO not only modulates splicing through $m^6Am$ removal but also binds directly to snRNAs, with its depletion impacting alternative splicing events. However, the mechanistic role of FTO and $m^6Am$ in splicing regulation remains largely unclear. Here, we demonstrate that FTO depletion leads to the accumulation of unprocessed snRNAs, whereas changes in $m^6Am$ levels do not directly influence snRNA 3'-end processing. Interestingly, we also find that FTO interacts with both snRNA 3'-end processing factors and snRNAs themselves, suggesting a broader role for FTO in multiple stages of snRNA biogenesis.

# 43
# Mechanisms of transcription attenuation and condensation of RNA polymerase II by RECQ5

**Marek Sebesta[1], Karel Skubnik[1], William Morton[1], Marek Kravec[2], Katerina Linhartova[1,3], Veronika Klapstova[1,3], Jiri Novacek[1], Karel Kubicek[1,3,4], Vitezslav Bryja[2], Robert Vacha[1,3,4], Richard Stefl[1,3]**

*1. CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czechia*
*2. Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czechia*
*3. National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czechia*
*4. Department of Condensed Matter Physics, Faculty of Science, Masaryk University; Brno, Czechia*

**E-mail of the presenting author:** marek.sebesta@ceitec.muni.cz

The elongation rates of RNA polymerase II (RNAPII) require precise control to prevent transcriptional stress, which can impede co-transcriptional pre-mRNA processing and contribute to many age- or disease-associated molecular changes (e.g., loss of proteostasis). Additionally mesoscale organization of transcription is thought to control the transcriptional rates and multiple factors have been reported to form biomolecular condensates and integrate RNAPII through the interaction with the C-terminal domain (CTD) of the largest subunit, RPB1. However, the structural organization of these condensates remains uncharacterized due to their small size and inherently dynamic nature. Here, we investigated the molecular mechanisms by which a general transcription factor – RECQ5 – associates with hyperphosphorylated RNAPII elongation complex (P-RNAPII EC) and controls translocation of RNAPII along genes. We combined biochemical reconstitution, electron cryomicroscopy, cryotomography, and coarse-grained simulations. We report two mechanisms by which RECQ5 modulates RNAPII transcription. At the atomic level, we demonstrate that RECQ5 uses the brake-helix as a doorstop to control RNAPII translocation along DNA, attenuating transcription. At the mesoscale level, RECQ5 forms a condensate scaffold matrix, integrating P-RNAPII EC through a network of site-specific interactions, reinforcing the condensate's structural integrity. Our integrative, multi-scale study provides insights into the structural basis of transcription attenuation and into the molecular architecture and biogenesis of a model RNAPII condensate.

# 44

# The phosphorylated trimeric SOSS1 complex and RNA polymerase II trigger liquid-liquid phase separation at double-strand breaks

**Qilin Long[1], Katerina Sedova[2], Vojtech Haluza[2], Adele Alagia[1], Zhichao Liu[1], Richard Stefl[2,3], Monika Gullerova[1], Marek Sebesta[2]**

*1. Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, United Kingdom*
*2. CEITEC-Central European Institute of Technology, Masaryk University, Brno, CZ-62500, Czechia*
*3. National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, CZ-62500, Czechia*

**E-mail of the presenting author:** 151266@muni.cz

The most toxic forms of DNA damage are double-strand breaks (DSBs). We have previously shown that RNA polymerase II (RNAPII), phosphorylated on tyrosine 1 (Y1P) on the C-terminal domain, transcribes RNA at DSBs to promote efficient DNA repair. However, it is still unknown how transcription is regulated at DSBs. Here, we show that the trimeric SOSS1 complex (hSSB1, INTS3, and c9orf80) binds to Y1P RNAPII in response to DNA damage, hSSB1 binds to R-loops, and formation of the SOSS1 complex assembly is required for the coexistence of replication protein A (RPA) and hSSB1 at DSBs. The damage-activated tyrosine kinase c-Abl phosphorylates hSSB1 to enable its binding to Y1P RNAPII and its recruitment to DSBs. Finally, we show both in vitro and in vivo that the SOSS1 complex and RNAPII form dynamic repair compartments at DSBs via liquid-liquid phase separation (LLPS). The loss of the trimeric SOSS1 leads to impaired DNA repair, highlighting its biological importance in the RNA-dependent DNA damage response.

# 45
# Analysis and Modulation of RNA Splicing in Retinal Dystrophy

**Carolina Silva**[1], **David Staněk**[1]

*1. Institute of Molecular Genetics of the Czech Academy of Sciences*

**E-mail of the presenting author:** carolina.silva@img.cas.cz

Retinitis pigmentosa (RP) is an inherited retinal disease characterized by progressive photoreceptor loss. Mutations in around 150 genes have been associated with RP, with alterations in Rhodopsin (*RHO*) and Pre-mRNA processing factor 31 (*PRPF31*) being the most frequent causes of autosomal dominant form of RP.

This study aims to investigate the effects of RP-associated mutations in these two genes on their splicing and to evaluate the potential of antisense oligonucleotide (ASO) therapy.

Seven RHO mutations and seven PRPF31 mutations, classified as pathogenic or of uncertain significance and associated with RP or other retinal dystrophies were selected from the ClinVar database. Mutations were inserted into reporter constructs and transfected into HEK cells to assess endogenous splicing.

Three of the seven mutations RHO mutations disrupted splicing. Two were located at canonical splice sites, while the third was found in intron 4. Of the seven PRPF31 mutations, three have been tested experimentally, and all disrupted splicing. Similarly, two were located at canonical splice sites and one in intron 10. Following confirmation of splicing disruption, ASOs were designed to correct these defects but their potential to modulate splicing was not confirmed experimentally.

Further research will focus on testing the remaining PRPF31 mutations and designing and evaluating ASOs for all identified mutations.

# 46
# From Stop to Start: A 40S-bound eRF1 ‘handover’ complex bridges termination to initiation

**Srinjoyee Singh pawar**[1], **Leoš Valášek**[1], **Jana Vojtová**[1], **Petr Těšina**[2]

*1. Laboratory of Regulation of Gene Expression, Institute of Microbiology of the Czech Academy of Sciences (IMIC)*
*2. Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic*

**E-mail of the presenting author:** psrinjoyee@gmail.com

eRF1 has the role of recognizing stop codons and catalyzing peptide release during translation termination. In the prevailing model, termination is followed by 60S–40S ribosomal subunit dissociation and recycling, with eRF1 released from the ribosome. However, our current results challenge this model. Using sucrose-gradient fractionation and eRF1 co-immunoprecipitation in HEK293T cells, we detected eRF1 across 40S and 80S ribosomal fractions, together with the ribosome-recycling ATPase ABCE1 and the 40S ribosomal protein RPS14. Strikingly, initiation-specific factors eIF1A, eIF2α, and eIF3A were also detected in complex with eRF1. And, by contrast, the elongation factor eEF1A (negative control) was absent. Altogether, our data indicate that eRF1 remains 40S-bound after termination, forming a post-termination handover complex that carries into early initiation and bridges termination to reinitiation.

# 47
# Uncovering the mechanisms of ALKBH5 demethylase in RNA biogenesis

**Jakub Sinsky**[1], **Praveenkumar Rengaraj**[1], **Veronika Rajecka**[1], **Matej Zabrady**[1], **Stepanka Vanacova**[1]

*1. Central European Institute of Technology (CEITEC), Masaryk University, Kamenice 5/E35, 625 00 Brno, Czech Republic*

**E-mail of the presenting author:** jakub.sinsky@ceitec.muni.cz

Epitranscriptome and its dynamics is emerging field in recent years revealing important associations with various human diseases, including cancer. Particularly, N6-methyladenosine (m6A) RNA modifications have been recently recognized as another crucial layer of regulation. While the recruitment and regulation of methyltransferases depositing the m6A marks are relatively well understood, the molecular mechanisms governing demethylases, including the ALKBH5, remains unclear. Dysregulation of ALKBH5, a key m6A demethylase, has been linked to defects in multiple stages of mRNA metabolism, including splicing, nuclear export, stability and translation, and more recently, also in cancer, particularly in acute myeloid leukemia (AML) and colorectal cancer. It has been proposed that the role of ALKBH5 in poor cancer prognosis relies in its overexpression, subsequent increased demethylation, and deregulation of target mRNAs of cancer-modulating genes. However, this is contradictory to the recent findings revealing that m6A abundance is primarily regulated by splicing efficiency and exon-junction complex deposition rather than ALKBH5 activity. Given its emerging role as a critical oncogenic factor in multiple cancers, including AML and colorectal cancer, understanding its precise molecular function is essential for uncovering new therapeutic opportunities in cancer treatment. In our project, we will utilize the state-of-the-art methods and pipelines for analysis of m6A- and ALKBH5-dependent mRNA-protein interactions and dynamics such as miCLIPseq, and 4sU RNA labelling coupled with PAR-CLIP enabling to identify time-resolved RNA-protein interactome. Using mRNA reporter construct mimicking an endogenous ALKBH5 target in degron-tagged and *ALKBH5*-knockout HEK239T cell lines, we will address the role of ALKBH5 in RNA biogenesis. Identified ALKBH5 targets will be experimentally validated in AML-like cell lineages MOLM13 and M-07e.

# 48
# The investigation of the role of non-canonical caps in RNA localization

**Irina Soldatova**[1], **Anton Škríba**[1], **Hana Cahová**[1]

*1. Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences*

**E-mail of the presenting author:** irina.soldatova@uochb.cas.cz

RNA modifications are essential for regulating RNA stability, function, and localization. One of the least explored RNA modifications is the 5' RNA cap. While the canonical m7G cap (cap1) is well-characterized, studies have revealed a growing diversity of non-canonical RNA caps. Among these, NAD and Ap4A caps have recently been identified. Ap4A acts as an RNA cap in mammalian cells and inhibits translation without affecting mRNA stability. This cap is cleaved by Nudt2, a Nudix family de-capping enzyme, but its cellular localization and regulation remain unclear. Recent findings also suggest that RNA cap structures influence subcellular localization of RNA.

Here we found that Nudt2 protein is presented in a cytoplasm of HEK293T cells and does not co-localize with mitochondria or peroxisomes. Also, we observe that Ap4A and cap1-capped mRNAs form cytoplasmic foci after cytoplasmic transfection. To further explore the role of non-canonical caps, we will deliver mRNA with different caps to cell nucleus and visualize their localization with super resolution microscopy. Additionally, we plan to follow co-localization of capped RNA with different cellular organelles (stress granules, p-bodies) and Nudt2 enzyme.

# 49
# The role of DIS3L2 in mitotic regulation

**Anna Vlčková**[1,2], **Ayat Masri**[1,3], **Štěpánka Vaňáčová**[1]

1. *CEITEC, Masaryk University*
2. *Faculty of Medicine, Masaryk University*
3. *Faculty of Science, Masaryk University*

**E-mail of the presenting author:** anna.hrckova@ceitec.muni.cz

DIS3L2 is a eukaryotic 3'–5' exoribonuclease and a homolog of the catalytic exosome subunit Rrp44 in yeast, as well as mammalian DIS3 and DIS3L. Unlike its paralogs, DIS3L2 specifically recognizes and degrades 3' oligouridylated RNAs, including structured RNA species, in a pathway dependent on terminal uridylyl transferases (TUT4/7), forming the TUT–DIS3L2 surveillance pathway.

Dysregulation of DIS3L2 has been implicated in diverse biological processes and pathologies, including apoptosis, viral infection, and cancer, and its loss is the genetic cause of Perlman syndrome. Moreover, absence of DIS3L2 leads to mitotic errors caused by chromosome mis-segregation and spindle pole defects.

Here, we apply endogenous proximity labeling (miniTurboID, mTID) to demonstrate that DIS3L2, TUT4, and TUT7 interact with a network of proteins essential for mitosis, notably components of the centromere, kinetochore, and centrosome. These findings suggest an unanticipated role of RNA processing in spindle assembly checkpoint regulation, highlighting a previously unrecognized link between RNA metabolism and mitotic control.

# 50
# In vitro selection of DNA-cleaving deoxyribozymes that differentiate methyl modifications in DNA

**Martin Volek**[1], **Claudia Höbartner**[1]

*1. Institute of Organic Chemistry, Julius-Maximilians-Universität Würzburg, Würzburg 970 70, Germany*

**E-mail of the presenting author:** martin.volek@uni-wuerzburg.de

DNA methylation is a key epigenetic modification involved in the regulation of gene expression, cell differentiation, and genome stability. Enzymes responsible for establishing and maintaining DNA methylation patterns, such as DNA methyltransferases (DNMTs), are important therapeutic targets, and the development of selective sensors for their presence is of high relevance. In this project we are using *in vitro* selection to isolate DNA-cleaving deoxyribozymes (catalytically active DNA molecules) that will be selectively activated by the presence of 5-methylcytidine ($m^5C$) or $N^6$-methyladenosine ($m^6A$) modifications in DNA. These DNAzymes will be further converted into fluorescence-based sensors for methyltransferase activity, enabling detection of DNMT3A/B or DnmA function. Our results will demonstrate the utility of synthetic functional DNA as a tool for epigenetic sensing and broaden the spectrum of high-throughput screening (HTS) assays which can identify novel small-molecule inhibitors of DNA methyltransferases.

*Acknowledgments: The work is supported by the Deutsche Forschungsgemeinschaft (DFG, awarded to C.H.) and by the Humboldt postdoctoral fellowship (awarded to M.V.).*

# 51
# Accuracy of Oxford nanopore technologies' direct RNA sequencing

**Marek Schwarz**[1], **Libor Krásný**[1], **Jana Wiedermannová**[1]

*1. Institute of Microbiology of the Czech Academy of Sciences, Prague, Czechia*

**E-mail of the presenting author:** jana.wiedermannova@biomed.cas.cz

We present an optimized workflow for Oxford Nanopore Technologies (ONT) direct RNA sequencing tailored to bacterial samples, incorporating a cost-effective DIY ribodepletion and enzymatic polyadenylation to maximize sequencing output for more accurate modification detection.

Our evaluation of data accuracy revealed a notable bias in RNA modification detection. Through systematic analysis, we identified the underlying cause of this bias related to current flow cell chemistry and basecalling algorithms. Based on these findings, we propose specific improvements to ONT's flow cell chemistry that could enhance modification calling accuracy. This work provides practical advancements for bacterial direct RNA sequencing and offers actionable recommendations for further technology development to improve epitranscriptomic analyses.

This work is supported by grant No. 24-10700S from the Czech Science Foundation.

# 52
# Splicing Aberrations in a Human Retinal Organoid Model Reveal a Photoreceptor-Intrinsic Mechanism for Retinitis Pigmentosa

**Felix Zimmann[1,2], Poulami Banik[1,2], Jan Kubovčiak[1,2], Prasoon K. Thakur[1], Martin Čapek[1], Michal Kolář[1], Eva Hrubá[3], Robert Dobrovolný[4], Zuzana Cvačková[1], Tomáš Bárta[3], David Staněk[1]**

*1. Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic*
*2. Faculty of Science, Charles University in Prague, Czech Republic*
*3. Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
*4. Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic*

**E-mail of the presenting author:** felix.zimmann@img.cas.cz

Autosomal dominant retinitis pigmentosa (adRP) is a progressive retinal disease marked by photoreceptors and retinal pigment epithelium (RPE) degeneration. While adRP is primarily a retinal disorder, it can be caused by mutations in ubiquitously expressed splicing factors, such as PRPF8. The precise mechanism by which these mutations lead to a retina-restricted phenotype and whether photoreceptors or the RPE are the primary affected cells remains unknown.

To investigate this, we developed a human retinal organoid model to study the impact of the adRP-associated mutation Y2334N in *PRPF8* on the neural retina, independent of the RPE. We used CRISPR/Cas9 to introduce the Y2334N mutation into *PRPF8* in human induced pluripotent stem cells (iPSCs), which were then differentiated into three-dimensional retinal organoids.

We found that while the overall cellular composition of the mutant organoids was unchanged, the formation of photoreceptor outer segments was significantly impaired. Transcriptome analysis revealed that although there were only minor changes in differential gene expression, there was a notable impact on the splicing of genes critical for neural and retinal function. Our data indicate that splicing perturbations directly affect photoreceptors, suggesting a mechanism for the retina-restricted phenotype of adRP caused by *PRPF8* mutations.

# 53
# Multidirectional protein trafficking between organellar and cytosolic ribosomes: insights from nucleomorph-containing organisms

**Jan Říha**[1,2], **Ondřej Gahura**[1]

*1. Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic*
*2. Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

**E-mail of the presenting author:** jan.riha@paru.cas.cz

Plastid-bearing eukaryotes contain three types of ribosomes - cytosolic, mitochondrial and plastidial - and thus three homologs of >30 ribosomal proteins (RPs) that are present in all of these ribosomes. Our pan-eukaryotic survey of organellar RPs revealed that several RPs of mitochondrial origin replaced their counterparts in the plastid ribosomes in plants, and vice versa. Further, plant and algal organellar ribosomes contain paralogs of cytosolic RPs, together indicating the interribosomal exchange of RPs occurs frequently on an evolutionary timescale.

Intercompartmental substitution of RPs might be pronounced in algal organisms crypthophytes and chlorarachniophytes, which contain nucleomorphs, vestigial yet functional nuclei associated with secondary plastids. Thus, they possess four genomes and four gene expression machineries including ribosomes, two of eukaryotic and two of prokaryotic origin. We searched available nuclear, nucleomorph and organellar genomes from nucleomorph-containing species for RPs and performed their phylogenetic analysis and targeting prediction to infer the origin of RPs of all four types of ribosomes. We found two genes of mitochondrial RPs retained in nucleomorph genomes. They are targeted to plastid, where they possibly replaced the original plastidial RPs. Further, we documented that the RPs of ribosomes in the periplastidial compartment (PPC) responsible for synthesis of nucleomorph-encoded proteins are poorly conserved on sequence level, and some are encoded in the nuclear genome. Some RPs in PPC were likely replaced by paralogs of cytosolic or mitochondrial RPs and several, mostly eukaryote-specific, lost completely. Our results document overall divergence of the PPC ribosomes, including unprecedented reduction of eukaryotic elements, presumably as a consequence of the reduced nucleomorph gene content. Nucleomorph-containing organisms thus provide unapparelled opportunity to study trends and plasticity of evolution of ribosomes.

# 54
# Designing a potent and ultra-stable RNase inhibitor for new diagnostic and research applications

**Václav Navrátil[1], Miroslav Smola[1], David Jakubec[1], Daniel Pokorný[1], Eva Mlynarčíková[1], Blanka Husťáková[1], Jana Veselá[1], Ondřej Žítek[1]**

*1. DIANA Biotechnologies*

**E-mail of the presenting author:** ondrej.zitek@dianabiotech.com

Working with RNA is challenged by its low stability caused by ubiquitous RNases. RNA can be stabilized by addition of recombinant protein RNase Inhibitors, which are potent yet very unstable. The main cause is their rapid oxidation and subsequent loss of activity even under mild conditions. Moreover, they are usually too thermally unstable to survive temperatures of reverse transcription, further limiting their use.

We have employed multiple bioinformatics approaches to identify possible mutation sites of the human RNase inhibitor to improve oxidative and thermal stability of the inhibitor. We created a battery of tests to evaluate oxidative, thermal, storage and assay stability and activity in human samples. From side-by-side comparisons, we were surprised how unstable are the current inhibitors and by how much their properties can be improved by site directed mutagenesis. We created hundreds of new sequences in multiple iterations from which we gradually selected the best.

We have obtained a highly mutated RNase inhibitor with greatly improved oxidative, thermal, storage and assay stabilities. It retains all activity in strongly oxidative solutions, such as 2% hydrogen peroxide, where all other inhibitors are already completely inactive. It also survives weeks to months at room temperature without a reducing agent. Unlike other inhibitors, it remains fully active in buffers routinely used for RT-PCR. It is also the only protein inhibitor retaining all of its activity at or above 55°C in RT-PCR buffers and protects RNA in biological samples to at least 60°C. Moreover, it shows even increased activity in human bodily fluids compared to other RNase inhibitors. This inhibitor is thus more active and more stable than any other currently available protein inhibitor.

Thanks to its properties, this inhibitor enables new applications in diagnostics and research. It enables reliable direct RT-PCR in diagnostics and in research. Higher thermal stability and thus higher reverse transcription temperature improves cDNA yield for complex RNAs. Better protection of RNA will be also helpful in RNA for sequencing or in preparing long cDNAs. It will also improve the quality of RNA produced in *in vitro* transcription. Altogether, this inhibitor may improve a lot of different areas of work with RNA.