RNA Club 2023

ABSTRACT BOOK

ORGANIZED BY

BIOLOGY CENTRE CAS

Institute of Parasitology

October 27, 2023

Resort Olšina

RNA Club 2023

October 27, 2023, Olšina

www.rnaclub.eu

The abstracts are presented as originally submitted by the authors.

Organizers

Ondřej Gahura
gahura@paru.cas.cz

Zdeněk Paris
parda@paru.cas.cz

SPONSORS

PROGRAMME

8:30-10:00 Registration

10:00-11:45 Session 1 (Chair: Leoš Valášek)

10:00 Welcome

10:10 **Marek Šebesta**
Spatial organization of RNA polymerase II•RECQ5 transcriptional condensate

10:25 **Tania Sanchez-Quirante Ⓟ**
Modified RNA for translation and CRISPR-Cas studies

10:40 **Pavel Vopálenský**
Identification of m6A modification in viroid RNA using LC-MS and Nanopore direct RNA sequencing

10:55 **David Doležel, Vlastimil Smýkal**
EFLa-type neuropeptide mRNA trans-splicing in hemimetabolous insects

11:10 **Prasoon Kumar Thakur Ⓟ**
Unveiling non-Canonical introns and splicing mechanisms in marine diplonemid

11:25 **Martin Volek Ⓟ**
Aurora: a fluorescent deoxyribozyme for high-throughput screening

11:40 **Robert Beal**
Bio-Rad Laboratories, sponsor's presentation

11:45-13:30 Lunch break and poster viewing

12:00 Lunch

13:30-14:10 Keynote speaker: Sebastian Glatt
tRNAslational control of eukaryotic gene expression

14:10-15:20 Session 2 (Chair: Petr Svoboda)

14:10 **Richard Zelenka**
MGP, sponsor's presentation

14:15 **Kristína Jendruchová Ⓟ**
The impact of yeast Tma20/MCTS1, Tma22/DENR and Tma64/eIF2D on translation reinitiation/ribosome recycling

14:30 **Kamila Horáčková**
Transcription and translation of genes encoded by Virus Like Elements

14:45 **Liam Keegan**
An RNA editing-independent function of ADAR1 inhibits PKR activation in mice

15:00 **Dragana Vukič Ⓟ**
Understanding human ADAR1's roles in RNA metabolism and innate immune response via protein-protein interactions

15:15 **Vojtěch Andrle**, **Vojtěch Ledvina**
East Port, sponsor's presentation

15:20-16:40 Poster session with coffee and refreshments

16:40-17:20 Keynote speaker: Michal Kolář
Exploring ribosome dynamics through large-scale computer simulations

17:20-18:40 Session 3 (Chair: Hana Cahová)

17:20 **Filip Brázdovič Ⓟ**
The loss of ribosomal expansion segments in Saccharomycotina

17:35 **Julie Kovářová**
Mechanisms behind the non-canonical genetic code of *Blastocrithidia nonstop*

17:50 **Jiří Koubek**
Nascent chain folding and transient polysome rearrangements orchestrate co-translational protein complex assembly in bacteria

18:05 **Zuzana Poláčková Ⓟ**
INTS11 mutations in patients with a phenotype overlapping with BRAT1-related disorders

18:20 **Petr Svoboda**
Natural and genetically engineered mammalian canonical RNAi in vivo

18:35 **Student's Prize Voting, Concluding Remarks**

19:00-??:?? Buffet dinner, party with music

20:00 Young Scientists' Prizes announcement

Ⓟ indicates eligibility for the Young Scientists' Prizes

LIST OF POSTERS

Poster number	Poster title	Presenting author
P01 Ⓟ	Regulation of initiation of translation during T cell activation	**Adriana Šubrtová**
P02 Ⓟ	Assembly of yeast torpedo termination complex	**Alžbeta Dikunová**
P03 Ⓟ	Conformational diversity of peptide deformylase and Its implications for ribosome binding	**Aneta Hrádková**
P04 Ⓟ	More than meets the eye – a hiPSC-derived retinal organoid model for RP-linked mutations in splicing factors	**Felix Zimmann**
P05 Ⓟ	-Title-	**Filip Karásek**
P06	tRNA synthetases as potential RNA capping enzymes	**Jan Říha**
P07	Different mechanisms of stress granules dissolution	**Jana Vojtová**
P08 Ⓟ	Computer simulations of the bacterial ribosome using a coarse-grained model	**Josef Cikhart**
P09 Ⓟ	How flexibility of delta affects transcription in G+ bacteria	**Klára Mikesková**
P10 Ⓟ	Downregulation of eIF3d and eIF3e increases expression of ribosomal proteins	**Klára Pospíšilová**
P11 Ⓟ	Substrate prediction model of human NudiX enzymes	**Klára Viktorinová**
P12	Differentiation of hiPSC into RPE cells and characterisation of splicing factor mutants	**Luisa Howainsky**
P13 Ⓟ	Exploring the evolutionary stability of the peptidyl transferase center in ribosomes using computer simulations	**Martin Mašek**
P14 Ⓟ	The role of coilin in snRNP biogenesis	**Nenad Radivojević**
P15	Diadenosine tetraphosphate (Ap4A) serves as a 5’ RNA cap in mammalian cells	**Ondřej Nešuta**
P16 Ⓟ	Correcting splicing of Prpf31 in retinitis pigmentosa - a step towards finding a cure	**Poulami Banik**
P17 Ⓟ	Assembly factor mtSAF24 in the biogenesis of small mitoribosomal subunit in trypanosomes	**Prashant Chauhan**
P18 Ⓟ	Rifampicin: stops or stimulates transcription	**Tamara Balgová**
P19 Ⓟ	Reactivation of RNA interference pathway in mammals	**Valeria Buccheri**
P20 Ⓟ	Novel transcription factors in *Bacillus subtilis*	**Veronika Kočárková**
P21 Ⓟ	Stops making sense - lessons from the tRNA anticodon stem	**Petra Miletínová**
P22	Downregulation of eIF3d and eIF3e decreases expression of key components of the activated MAPK/ERK signaling pathway	**Anna Herrmannová**

Ⓟ indicates eligibility for the Young Scientists’ Prizes

TALKS

Session 1

Spatial organization of RNA polymerase II•RECQ5 transcriptional condensate

Marek Sebesta[1], Karek Skubnik[1], William Morton[1], Marek Kravec[2], Katerina Linhartova[1,3], Veronika Klapstova[1,3], Jiri Novacek[1], Karel Kubicek[1,3,4,5], 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*

[5]*Institute of Molecular Genetics of the Czech Academy of Sciences, v.v.i.; Prague, Czechia*

RNA polymerase II (RNAPII) is thought to shuttle between transcription-associated condensates in a phosphorylation-dependent manner, but the architecture of these RNAPII condensates remains unknown. To visualize it, we used cryo-electron tomography, coupled with molecular dynamics simulations to study a heterotypic condensate between hyperphosphorylated RNAPII elongation complex (RNAPII EC) and a general elongation factor RECQ5, which are colocalized in liquid droplets *in vivo*, *in vitro*, and *in silico*. RECQ5 forms a condensate scaffold and integrates RNAPII EC through interactions with hyperphosphorylated RNAPII C-terminal domain and binds the surface of RNAPII's jaw to contact downstream DNA entering the RNAPII active center. Together, our results provide an integrative understanding of RNAPII condensate assembly and lay down the basis for future studies of more complex cellular condensates using tomography.

Session 1

Modified RNA for translation and CRISPR-Cas studies

Tania Sanchez-Quirante[1,2], Erika Kužmová[2], Michal Hocek[1,2]

[1]Institute of Organic Chemistry and Biochemistry Czech Academy of Sciences. Flemingovo nam.2, 16610 Prague 6, Czech Republic
[2]Department of Organic Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 12843 Prague 2, Czech Republic

The study of RNA has increased considerably in the recent years. It is known that some modifications in the RNA improve its biological effects, as increased stability and thus having consequences in translation efficiency or in CRISPR-CAS experiments. Previously our lab reported the efficiency of the T7 polymerase to incorporate different small modifications. In this work, a library of 8 nucleoside triphosphates, with methyl and ethyl modification, have been synthetized and incorporated during in-vitro transcription by the T7 polymerase. Different length of the templates has been used to synthetize short RNAs such as 35nt or longer as 70nt long. Single-guide RNA 99nt long have been synthetized and used for CRISPR-Cas and stability experiments in Human serum. On the other hand, mRNA was synthetized using pJET_T7_Gluc_128A plasmid digested with restriction enzyme AarI as a DNA template, and evaluated the translation efficiency of Gaussia Luciferase in HeLa S3 cells.

Session 1

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

Pavel Vopalensky[1], Lucia Duricekova[1], Ondrej Luksan[1], Francesco di Serio[2], Beatriz Navarro[2] and Hana Cahova[1]

[1]IOCB Prague, Flemingovo namesti 542/2, Prague, Czech Republic
[2]Institute for Sustainable Plant Protection, National Research Council, Bari, Italy

Viroids are small circular non-coding RNAs that act as infectious pathogens in higher plants causing serious damage in agriculture. Despite viroid RNAs do not code for any proteins, viroids can autonomously replicate in plant cells. The replication can occur either in the nucleus (family Pospiviroidae), or in the chloroplasts (family Avsunviroidae). The structural features of viroid RNA determining the subcellular sorting into these two organelles are not well understood, however, RNA modifications were suggested to play a role. Here, we set out to identify the m6A RNA modifications in the Citrus exocortis viroid (CEVd, Pospiviroidae) and the Avocado sunblotch viroid (ASBV, Avsunviroidae) using LC-MS and Oxford Nanopore Technology (ONT) direct RNA sequencing approaches. Using LC-MS analysis we detected m6A and ribose methylated nucleosides in viroid RNA. While we were not able to detect m6A modification in the ASBV RNA, the CEVd (Pospiviroidae) RNA contained one m6A modification per molecule of RNA. To precisely allocate the m6A modification into the structural context of the CEVd viroid RNA, we have implemented a protocol for ONT direct RNA sequencing of viroid RNA. Using several available algorithms for m6A detection in direct RNA reads, we identified candidate m6A sites and we are currently confirming these sites by biochemical methods. Once confirmed, we plan to perform in vivo functional studies to reveal the role of m6A modification in CEVd viroid biology.

Session 1

EFLa-type neuropeptide mRNA trans-splicing in hemimetabolous insects

Vlastimil Smykal[1], Bulah Chia-Hsiang Wu[1], Daniela Chvalova[1], Marketa Hejnikova[2], Martin Lichy[1], Ping Chen[2] , David Dolezel[1]

[1] Biology Centre CAS, Institute of Entomology, Ceske Budejovice, Czech Republic
[2] Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

EFLa-type neuropeptides are present in many arthropods, including several insect orders. Besides EFLa's elusive function, the way the mature *EFLa*-encoding transcripts are formed is also enigmatic, as *EFLa* exons spliced to exons of *Proh-4* gene were found in *Pyrrhocoris apterus* (Heteroptera), *Bemisia tabaci* (Sternorrhyncha) and *Locusta migratoria* (Orthoptera). The presence of intergenic *Proh-4/EFLa* fused transcripts can be theoretically explained by canonical cis-splicing, cis-splicing of adjacent genes (cis-SAG), or even by trans-splicing, a rare molecular mechanism in insects. We focused on *P. apterus*, where *Proh-4/EFLa* transcripts represent about 1/10 of *Proh-4* expression, *EFLa* pre-mRNAs lack in-frame translation start site, and where gene-linkage and genome analyses excluded cis-splicing and cis-SAG mechanism of *Proh-4/EFLa* generation. Trans-spliced *Proh-4/EFLa* transcripts were detected by full-length mRNA sequencing using Oxford Nanopore technology with isoform-specific resolution. In-silico analysis of 3’ splice site (3’ss) strength predicted weak 3’ss of *Proh-4* intron 2, permitting trans-splicing with 10-fold stronger *EFLa* outron 3’ss. Analogous analysis in *L. migratoria* found less profound *Proh-4* to *EFLa* 3’ss strength difference, reflected in 10-fold lower *Proh-4/EFLa* trans-splicing, determined by quantitative real-time PCR. We conclude that although the exact trans-splicing mechanism generating *Proh-4/EFLa* transcripts stays elusive, it can represent conserved and sound gene expression mechanism.

Session 1

Unveiling Non-Canonical Introns and Splicing Mechanisms in Marine Diplonemids

Prasoon Kumar Thakur[1], Filip Karásek[1], Anzhelika Butenko[2], Ales Horak[2], Julius Lukes2, and David Staněk[1]

[1]Institute of Molecular Genetics of the CAS, v. v. i, Prague, Czech Republic
[2]Institute of Parasitology, Biology Centre of the CAS, v.v.i., and University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic

RNA splicing plays a critical role in regulating gene expression and transcriptome diversity in a variety of eukaryotes. Recent global surveys of marine biodiversity revealed that planktonic diplonemids are amongst the most abundant and diverse marine organisms. These so-far overlooked protists related to important kinetoplastid parasites (Trypanosoma, Leishmania) as well as ubiquitous prospective biofuel producers (Euglena) have unusual splice site and lack the canonical intron (GU-AG). This suggests the existence of an alternative mechanism of intron removal and implies diplonemids can be an excellent model for the study of intron evolution. We have therefore sequenced genomes and transcriptomes of six marine diplonemids. We have assembled the genome and transcriptome of all five diplonema. Further, We have be carried out comprehensive splice-site analysis of these species. Firstly, we identified non-canonical introns and illustrated their common sequence and/or structural features. Moreover, our analyses revealed presence of cannonical Spliceosomal components. Overall, current study showed general mechanisms and pattern of RNA splicing and compare it to the canonical eukaryotic model.

Session 1

Aurora: a fluorescent deoxyribozyme for high-throughput screening

Martin Volek[1, 2]; Jaroslav Kurfürst[1, 3]; Matúš Drexler[1]; Michal Svoboda[1]; Pavel Srb[1]; Václav Veverka[1, 4] and Edward A. Curtis[1]

[1]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague 166 10, Czech Republic;
[2]Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Prague 128 44, Czech Republic;
[3]Department of Informatics and Chemistry, University of Chemistry and Technology, Prague 166 28, Czech Republic;
[4]Department of Cell Biology, Faculty of Science, Charles University in Prague, Prague 128 44, Czech Republic

Fluorescence facilitates detection, visualization, and tracking of molecules with high sensitivity and specificity. A functional DNA molecule that generates a fluorescent signal would offer significant advantages for many applications compared to intrinsically fluorescent proteins (which are expensive and labor intensive to synthesize) and fluorescent RNA aptamers (which are unstable under most conditions). Here we describe a novel deoxyriboyzme that rapidly and efficiently generates a stable fluorescent product using an inexpensive and commercially available substrate. An engineered version can detect picomolar concentrations of ribonucleases in a simple homogeneous assay, and was used to identify potent inhibitors of the SARS-CoV-2 ribonuclease Nsp15 in a highthroughput screen. Our results expand the toolkit of functional DNA parts, and show how such tools can be used in applied research.

Keynote Lecture

tRNAslational control of eukaryotic gene expression

Sebastian Glatt[1]

[1]*Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, PL*

Session 2

The impact of yeast Tma20/MCTS1, Tma22/DENR and Tma64/eIF2D on translation reinitiation/ribosome recycling

Kristína Jendruchová[1], Stanislava Gunisova[1], Leos S. Valasek[1]

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

Yeast Tma22/Tma20 and Tma64 proteins are homologues to known mammalian translation reinitiation and ribosome recycling factors DENR/MCTS1 and eIF2D. They have been shown to promote 40S subunit recycling in vivo in yeast, influenced by penultimate codon identity of the translated ORF. We use an established reporter system based on the yeast GCN4 gene, known for its tightly regulated translational control based on translation reinitiation, to examine the impact of the Tma proteins on downstream reinitiation after short uORFs, and 40S subunit recycling. We also investigated the influence of Tma20/22/64 on reinitiation after very short uORFs comprised of a start and stop codon, which has been proven dependent on DENR in mammals. We found that unlike their homologues in higher eukaryotes, these Tma proteins function in a manner more in line with general 40S recycling rather than reinitiation and recycling.

Session 2
Transcription and translation of genes encoded by Virus Like Elements

Kamila Horáčková[1], Kristýna Ľalíková, Priyang Singhvi, Michal Sýkora, Kristina Roučová, Martin Pospíšek, Václav Vopálenský
[1]Laboratory of RNA Biochemistry, Department of Genetics and Microbiology, Faculty of Science, Charles University, Vinicna 5, Prague 2, 128 44, Czech Republic

Linear yeast plasmids, which represent a unique and complex system, deserve to be thoroughly studied due to their exclusive characteristics. These plasmids are localized exclusively in the cytoplasm, have a highly compacted linear genome with inverted terminal repeats at the ends of genome DNA and a significant content of A/T base pairs, and operate independently of the host replication and transcription apparatus. These properties not only make them challenging to study, but also puts them in the position of a potential model for viruses with similar properties, especially large nucleocytoplasmic DNA viruses present exclusively in the cytoplasm of infected cells, such as poxviruses. A prominent example of linear yeast plasmids are those of the yeast *Kluyveromyces lactis*, known as pGKL1 and pGKL2. Our research focuses on the transcription apparatus encoded by linear plasmids, including the RNA polymerase holoenzyme, the capping enzyme, the helicase and other, as yet unknown, components of the transcription apparatus. We have produced components of the transcription apparatus in homologous and in different heterologous expression systems to reconstruct a functional complex, which we subsequently plan to analyse by cryoelectron microscopy. Previous findings from our research group indicate that the majority of mRNAs transcribed by the pGKL-specific transcription apparatus exhibit a unique structure. They possess a relatively short 5' untranslated region containing almost exclusively non-templated adenosine residues, directly followed by an AUG initiation codon. Surprisingly, plasmid-specific mRNAs lack the 7-methylguanosine cap necessary for classical translation initiation in eukaryotic cells. This characteristic organization of plasmid-specific mRNAs is reminiscent of the intermediate and late mRNAs transcribed by the RNA polymerase of vaccinia virus, a representative of poxviruses. Our next goal was to elucidate the mechanism of translation initiation of these mRNAs. For this purpose, we developed a set of reporter plasmids that allowed us to investigate the effect of different numbers of adenosine residues on the level of translation. The first group of reporters was derived from natural, cytoplasm-localized pGKL plasmids, yielding mRNAs with a 5' poly(A) non-templated sequence and lacking the 7-methylguanosine cap. In contrast, the second set of reporters was derived from a yeast nuclear 2μ plasmid, which allowed us to determine the effect of different lengths of 5′ poly(A) sequences bearing a 7-methylguanosine cap on the translation rate of these mRNAs. Taking together, we successfully developed and partially tested a reporter system to determine the effect of the 5' poly(A) sequence on translation rate; determined the strength of promoters directing the expression of pGKL-specific mRNA molecules and finally, we purified key components of pGKL-specific RNA polymerase.

Supported by the project National Institute of virology and bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU and by the Grant Agency of Czech Republic (GACR 21-25504S).

Session 2

An RNA editing-independent function of ADAR1 inhibits PKR activation in mice

Ketty Sinigaglia[1], Anna Cherian[1], Dragana Vukic[1], Quopei Du[1], Janka Melicherova[1], Pavla Linhartova[1], Lisa Zerad[2], Stanislav Stejskal[1], Radek Malik[3], Jan Prochazka[4], Nadège Bondurand[2], Radislav Sedlacek[4], Mary A. O'Connell[1*] and Liam P. Keegan[1*]

[1]Central European Institute for Technology at Masaryk University (CEITEC MU), Building E35, Kamenice 735/5, Brno, CZ 62500, Czechia.
[2]Laboratory of Embryology and Genetics of Human Malformation, Imagine *Institute, INSERM UMR 1163, Université de Paris Cité, F-75015, Paris, France.*
[3]Laboratory of Epigenetic Regulation, Institute of Molecular Genetics of the Czech Academy of Sciences, Vídeňská 1083, CZ 142 20, Praha 4, Czechia.
[4]Institute of Molecular Genetics of the Czech Academy of Sciences, Prumyslova 595, 252 50 Vestec, Czechia.

The deamination of the adenosine base to inosine in dsRNA is catalyzed by the ADAR RNA editing enzymes. In mammals, there are two active ADAR enzymes; ADAR1 and ADAR2. The editing activity of ADAR1 is essential for the discrimination of self and non-self RNA by the innate immune cytoplasmic dsRNA sensors MDA5 and RIGI that signal downstream to MAVS. In humans, mutations in ADAR1 that decrease RNA editing activity cause Aicardi Goutières Syndrome (AGS). *Adar* null mutant mice are embryonic lethal and die by E12.5. Both conditions are characterized by high aberrant interferon induction. We hypothesized that since *Adar, Mavs* double mutant mice survive till birth but die as pups, usually within 14 days, other innate immune dsRNA-driven pathways must be in play. *Adar* null mouse embryos have an increased expression of Pkr (*Eif2ak2*), a protein kinase that is activated by unedited dsRNA. We show that the early death of the *Adar, Mavs* double mutant pups and severe gut defects arising from death of proliferating gut stem cells stem and their aberrant differentiation are rescued in *Adar, Mavs, Eif2ak2* triple mutant mice. We also report a direct regulatory interaction between PKR and ADAR1 that inhibits the phosphorylation of the PKR kinase domain and activation of PKR. Using ADAR1 mutants expressed in cells, we show that ADAR1 interacts through its third dsRNA binding domain (RBDIII) with PKR, in an editing-independent manner. Our results indicate that in addition to dsRNA editing, ADAR1 protein-protein interactions regulate the innate immune response.

Session 2

Understanding human ADAR1's roles in RNA metabolism and innate immune response via protein-protein interactions

Dragana Vukič[1], Anna Cherian[1], Rita Bong[2], Salla Keskitalo[2], Liam Keegan[1], Markku Varjosalo[2], and Mary A. O'Connell[1]

[1]Central European Institute for Technology at Masaryk University (CEITEC MU), Kamenice 735/5, Brno, CZ 62500, Czechia
[2]Institute of Biotechnology, HiLIFE Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland

Inosine, one of the most abundant post-transcriptional RNA modifications, is generated via hydrolytic deamination of adenosine (RNA editing) within double-stranded (ds)RNA. This process is mediated by the adenosine deaminases acting on a dsRNA (ADARs) family of enzymes. Inosine can impact RNA structure and function, including coding effects; thus, it plays an essential role in various cellular processes. Furthermore, the presence of inosine in dsRNA serves as an important marker of cellular "self" dsRNA and it is critical for preventing aberrant activation of IFN response. In mammals, the ADAR1 protein is expressed as two isoforms: a constitutive isoform predominantly found in the nucleus (p110) and an isoform induced by interferon (p150) that is primarily located in the cytoplasm. Interestingly, ADAR1 has a multifaceted impact that goes beyond its RNA editing activity and is gaining increased recognition. To delve deeper into the editing-independent and isoform-specific functions of ADAR1, we generated an inducible Flp-In-293 stable cell line that overexpresses both p150 and p110 isoform. We then investigated protein-protein interactions using two techniques: BioID to enrich the more transient interactions and StrepII tag-based immunoprecipitation for stable interactors. Finally, we examined these interactions under non-treated and interferon-induced states. Multiple common complexes were identified across all datasets. These complexes ranged from rRNA and mRNA processing factors to some IFN-related proteins, broadening our understanding of ADAR1's involvement in multiple steps of RNA metabolism and IFN signaling.

Keynote Lecture

Exploring ribosome dynamics through large-scale computer simulations

Michal Kolář[1]

[1]*Department of Physical Chemistry, University of Chemistry and Technology, Prague*

The ribosome is renowned as a ubiquitous nanomachine responsible for protein synthesis in all known forms of life. Composed of a few strands of RNA and a few dozen ribosomal proteins, ribosome substantial size and complexity leaves numerous gaps in our understanding of its functional dynamics. This is where computer simulations may step in, offering a virtual laboratory that allows researchers to observe and manipulate ribosome as a whole or its constituent parts in silico [1]. The talk will outline the conceptual advances that paved the way to the first simulations of the ribosome, encompassing over two million atoms. Several published [2, 3] and ongoing case studies will be presented. Attention will be paid to the ribosome exit tunnel, approximately 10 nm long structural element present in all ribosomes, which plays a critical role in ribosome regulation.

[1] Bock et al. 2023, PMID: 36719969
[2] Kolář et al. 2022, PMID: 35150281
[3] McGrath et al. 2022, PMID: 36335428

Session 3

The loss of ribosomal expansion segments in Saccharomycotina

Filip Brázdovič[1,4], Peter Baráth[2], Bronislava Brejová[3], Barbara Siváková[2], Jozef Nosek[1]

[1]Department of Biochemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Slovakia,
[2]Institute of Chemistry, Slovak Academy of Sciences, Slovakia,
[3]Department of Computer Science, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia, [4]Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

Ribosomes are protein synthesizing machines highly conserved in all three domains of life. They are composed of tens of proteins and a set of ribosomal RNA (rRNA) molecules. While the core ribosomal functions remain the same in all organisms, comparative studies revealed numerous structural differences. When comparing bacteria, yeasts and mammals, the ribosome sizes positively correlate with the genome complexity. This is mainly due to the presence of rRNA regions termed expansion segments (ESs) protruding from the surface of the ribosomes. ESs were found to be involved in ribosome biogenesis, translation, and co-translational protein modification. Their deletions usually affect the cell growth pointing to their importance, although the roles of individual ESs remain elusive. Here, we analyzed the rRNAs of the yeasts from the subphylum Saccharomycotina. We found that several yeasts lack more than 450 and 150 nt from the 25S and 18S rRNA, respectively. This reduction is unrelated to the genome size and occurred independently in species belonging to different phylogenetic lineages. In silico and LC-MS/MS analyses show that ribosomal proteins are conserved suggesting that the reduction is limited to rRNA. In all species, the missing regions correspond to the ESs. This represents the shift toward ancestral rRNA structure.

Session 3

Mechanisms behind the non-canonical genetic code of *Blastocrithidia nonstop*

Julie Kovářová[1], Zdeněk Paris[1]

[1]*Institute of Parasitology, Biology Centre, České Budějovice, Czech Republic*

Blastocrithidia nonstop is a unicellular parasite from the Euglenozoa group that has all three stop codons reassigned to code for amino acids. For UAG and UAA, cognate tRNAs-Glu are present in the genome. For UGA, however, there is no such tRNA, but it is translated by a tRNA-Trp with a shortened anticodon stem. In general, the anticodon stem consists of 5 base pairs, but in *B. nonstop* it is only 4 base pairs long. Our tRNA sequencing revealed that half of the 4-bp tRNA-Trp population is edited from cytidine to uridine at position 19. Surprisingly, this single change, distant from the anticodon, completely abolishes the ability to read through the UGA stop codon. We hypothesize that this may serve as a regulatory mechanism for the translation of genes containing in-frame stop codons. Based on bioinformatic analyses of the genome, multiple UAAs are required at the end of each gene to act as a genuine stop codon. To validate this prediction, we will perform ribosome profiling. This will allow us to determine the dynamics of reassigned codons readthrough and also if termination at any of these UAAs is random, or if their number or position play specific roles. Overall, we conclude that the obscure parasite *B. nonstop* is becoming a model organism for translation and can provide us with novel solutions and mechanisms that were previously inconceivable.

Session 3

Nascent chain folding and transient polysome rearrangements orchestrate co-translational protein complex assembly in bacteria

Jiří Koubek[1], Sebastian Filbeck, Jaro Schmitt, Santiago Maya, Frank Tippmann, Günter Kramer, Stefan Pfeffer, Bernd Bukau

[1]ZMBH, Heidelberg University, Germany

Proteins mostly function as part of oligomeric complexes, necessitating efficient assembly of newly synthesized proteins for cellular functionality. Recent evidence suggests that in eukaryotic cells protein complexes often assemble co-translationally. However, fundamental aspects of co-translational assembly remain poorly understood, particularly regarding how translation coordinates with folding and assembly. Additionally, it is uncertain whether polysomes adopt a conformation specific to co-translational assembly, as the canonical polysomal architecture minimizes contacts between nascent chains.
To address these questions, we employed a range of techniques, including ribosome profiling, disome-selective profiling, fluorescence spectroscopy, cryo-electron microscopy, and cryo-electron tomography. Our study focused on the assembly of a model dimeric *E. coli* protein with a well-characterized N-terminal dimerization domain. We observed that co-translational folding of the N-terminal PheA dimerization domain initiates when the N-terminus is still in the ribosome exit tunnel and achieves a native fold upon dimerization. Nascent chain then mediate interaction of two translating ribosomes by placing the peptide exit tunnels in close proximity. Nevertheless, this rearrangement is only transient, as the architecture is dictated by mRNA. Overall, our work provides the first evidence that polysomes adapt their structure according to the nature of the translated nascent chain and that assembly and folding are highly interconnected. These findings significantly advance our understanding of how cells coordinate the co-translational formation of protein complexes and shed light on the structural organization of this process.

Session 3

INTS11 mutations in patients with a phenotype overlapping with BRAT1-related disorders

Zuzana Poláčková[1], Aveeva Herold[2], Denisa Bronišová[1], Kamila Burdová[1], Cyril Bařinka[3], Eric Samarut[2], Reza Maroofian[4], Hana Hanzlíková[1,5]

[1]Laboratory of Genome Dynamics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Neuroscience, Université de Montréal, Montreal, QC, Canada
[3]Laboratory of Structural Biology, Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic
[4]Department of Neuromuscular Diseases, University College London, London, United Kingdom
[5]Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Switzerland

Our recent research has suggested the critical role played by the BRAT1/INTS9/INTS11 complex in the intricate realm of brain development and maintenance. This dynamic assembly comprises BRAT1 and a catalytic RNA endonuclease heterodimer, formed by the INTS9 and INTS11 subunits of the Integrator complex. The Integrator complex is responsible for precisely processing of various non-coding RNAs and pre-mRNAs, thus shaping the fundamental machinery of cellular function. While BRAT1 is a protein encoded by a gene that is expressed ubiquitously, germline alterations in BRAT1, especially, lead to various neurological syndromes characterized by both neurodevelopmental delay and neurodegeneration. Similarly, several Integrator subunits, including INTS11, have been found to be mutated in neurological syndromes. In this study, we describe novel biallelic mutations in the human INTS11 gene, and confirm the neuropathology in an Ints11-deleted zebrafish model. Intriguingly, the phenotype observed in INTS11 patients substantially overlaps with that of individuals harboring BRAT1 mutations, emphasizing a common pathological basis. We demonstrate that cells from affected individuals, whether they have INTS11 or BRAT1 mutations, exhibit defects in the processing of small nuclear RNAs (snRNAs) and accumulate unprocessed U1 snRNAs in the nucleus, that correlate with the severity of the disease. Notably, our preliminary data suggest that the BRAT1 loss may impact INTS11 stability and/or its turnover on the RNA substrates. In conclusion, our study provides critical insights into the molecular mechanisms underlying neurological disorders associated with INTS11 and BRAT1 mutations. These findings offer the development of diagnostic markers and potential therapeutic avenues for related neurological disorders.

Session 3

Natural and genetically engineered mammalian canonical RNAi in vivo

Petr Svoboda[1]

[1]*Institute of Molecular Genetics, CAS, Prague*

RNA interference (RNAi) has been defined as sequence-specific mRNA degradation induced by long double-stranded RNA (dsRNA). RNAi is an ancient eukaryotic post transcriptional silencing pathway providing gene silencing and defense against viruses and mobile elements. In this sequence-specific system, RNase III Dicer recognizes long double stranded RNA and cuts it into ~22nt short interfering RNAs (siRNA), which guide elimination of RNAs with complementary sequences. In mammals, RNAi became a vestigial pathway mammalian Dicer is structurally adapted to generate microRNAs (miRNAs), a different class of small regulatory RNAs. However, RNAi became re-activated in rodent oocytes because of a long terminal repeat (LTR) insertion in the common ancestor of mice and hamsters. This LTR insertion gave rise to expression of an N-terminally truncated oocyte-specific Dicer isoform, which is efficiently producing siRNAs. Remarkably, evolution of endogenous RNAi in oocytes in house mouse, Norwegian rat, and golden hamster yielded remarkably different outcomes. Our analysis of mouse and rat mutants lacking the truncated Dicer variant provides insights into principles governing evolution of RNAi roles in mammalian oocytes and finally puts older mouse data into experimentally supported evolutionary context. Furthermore, we also genetically engineered mice to reactivate RNAi in vivo through conversion of the endogenous Dicer gene into a variant expressing a truncated Dicer variant (ΔHEL1) supporting RNAi. We show that homozygosity of the ΔHEL1 variant is perinatally lethal because of infidelity of miRNA biogenesis. At the same time, a single ΔHEL1 allele is well tolerated, increases efficiency of siRNA production in vivo, and can support functional endogenous RNAi. Finally, experiments with viral infections of genetically engineered mice revealed that the ΔHEL1 variant was able to provide significant antiviral effect against one of the four tested viruses.

POSTERS

P01

Regulation of initiation of translation during T cell activation

Adriana Subrtova[1], Adriana Roithova[1], Leos Valasek[1]

[1]*Institute of Microbiology of the CAS, Czech Republic*

T-lymphocytes coordinate many aspects of adaptive immunity throughout life, including responses to pathogens, allergens, and tumors. If T-lymphocytes are not precisely regulated, they can also become dangerous for us and give rise to a super tumor disease - lymphoma. A greater understanding of these cells from their origin to their differential is essential for the diagnosis and treatment of these types of diseases. During the T cell activation, a dramatic increase in protein synthesis must be precisely controlled and regulated. Stimulation of translation is due to an increase in the rate of initiation caused by regulation of the activities of initiation factors (eIFs). In our project, we will study the dynamics of eIFs by Sel-TCP-seq and focus on the molecular mechanism of (eIFs) during T-cell activation and after anti-cancer drug treatment of the lymphoma T-cell line. Previous studies have shown a link between METTL3 /METTL14 methylases with T cell homeostasis and differentiation. We also aim to unravel the role of internal markers of m6A and related factors during T-cell activation.

P02

Assembly of yeast torpedo termination complex

Alžbeta Dikunová[1], Nikola Nosková[1], Tomáš Klumpler[1], Martin Polák[1], David Zapletal[1,2] and Richard Štefl[1]

[1]CEITEC–Central European Institute of Technology, Masaryk University, Brno, CZ-62500, Czech Republic
[2]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, CZ-62500, Czech Republic

Transcription termination is essential for delineating the genetic information stored in DNA, as it establishes the boundaries of transcriptional units[1]. In yeast, there are two model pathways how termination of mRNA coding genes is organized: allosteric and torpedo models. The torpedo model considers that the unprotected free 5'-end of the mRNA transcript is digested by nuclease until it collides with RNA polymerase II (RNAPII), leading to dissociation from the template.

Studies in the yeasts showed that exposed free 5'-end of RNA serves as an entry point for Rat1, a 5'-3' exonuclease. Stimulated by its cofactor Rai1, the Rai1/Rat1 (RR) complex greatly stimulates spontaneous termination[2]. However, the exact mechanism of how the RR complex is recruited to the site of transcription and how RNAPII is released from the DNA is unknown. Findings also suggest that the torpedo complex is recruited by Rtt103, recognizing Ser2[3] and or Thr4 phosphorylation marks of RNAPII. This allows us to hypothesize that Rtt103 recruits the 5'-3' RNA termination machinery. We set out to investigate the structure of the torpedo complex in *S. cerevisiae.* Our 3.23*Å*-cryo-EM structure of RR reveals structural insights into the Rai1/Rat1 complex association. Next, our integrative model combining cryo-EM, XL-MS, small-angle X-ray scattering, size exclusion chromatography and binding affinities (FA) suggests how RR is recruited through the Rtt103 adaptor to the site of transcription.

1. Ray-Soni, A., Bellecourt, M. J. & Landick, R. Mechanisms of Bacterial Transcription Termination: All Good Things Must End. *https://doi.org/10.1146/annurev-biochem-060815-014844* **85**, 319–347 (2016).
2. Han, Z. *et al.* DNA-directed termination of RNA polymerase II transcription. *Mol Cell* **83**, 3253-3267.e7 (2023).
3. Nagarajan, V. K., Jones, C. I., Newbury, S. F. & Green, P. J. XRN 5’→3’ exoribonucleases: Structure, mechanisms and functions. *Biochim Biophys Acta* **1829**, 590 (2013).

P03

Conformational Diversity of Peptide Deformylase and Its Implications for Ribosome Binding

Aneta Hrádková[*1], Jiří Kubíček, Michal H. Kolář

[1]*Institute of Molecular Genetics, Czech Academy of Sciences*

The first enzyme that bacterial proteins encounter after their birth is the peptide deformylase (PDF). PDF binds to the ribosome surface and removes the formyl group from the N terminus of the nascent protein, which emerges from the ribosomal exit tunnel. There are two distinct types of PDFs, primarily differing in their C-termini. Type I PDFs feature a C-terminal α-helix that serves as the connection point between the PDF's catalytic domain and the ribosome's surface. Conversely, Type II PDFs exhibit an intrinsically disordered C-terminal region and the mechanism by which Type II PDFs bind to the ribosome is unknown. Moreover, secondary structure propensities of the Type II PDF C terminus remain elusive. In our study, we investigate the folding behavior of the C-terminal region by conducting all-atom molecular dynamics simulations of PDFs derived from various organisms, as well as simulations of the isolated C-terminal fragments in water. Our findings reveal significant differences in the conformational ensembles of the C-termini between Type I and Type II PDFs. We quantified the secondary structure propensities of the simulated systems and found notable differences as well. The catalytic domain of PDF plays a pivotal role in shaping the C-terminal fragments. In our ongoing research, we aim to provide a detailed molecular description of the interaction interface between Type II PDFs and the bacterial ribosome. Our results shed light on the potential binding mode of PDF to the ribosomal surface.

P04

More than meets the eye – a hiPSC-derived retinal organoid model for RP-linked mutations in splicing factors

Felix Zimmann[1]*, Zuzana Cvackova[1], Tomas Barta[2], David Stanek[1]

[1]Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Histology and Embryology,
Faculty of Medicine, Masaryk University, Brno, Czech Republic.
**felix.zimmann@img.cas.cz*

Retinitis pigmentosa (RP) is the primary cause of hereditary blindness. It is characterized by a progressive deterioration of photoreceptors and remarkable clinical and genetic diversity. While most RP-linked mutations are found in genes critical to photoreceptor function, some unexpectedly affect ubiquitous splicing factors like PRPF8. Hitherto, how these mutations lead to RP remains is still not fully understood. Here, we present the establishment of a human retinal organoid model to elucidate the fundamental disease mechanism of splicing factor mutations and their retina-specific effects. Using CRISPR-Cas9 gene editing, we introduced an RP-linked mutation into the PRPF8 regulatory Jab1/MPN domain in human induced pluripotent stem cells (hiPSC), which were then differentiated into three-dimensional retinal organoids. The cellular composition of retinal organoids from mutated hiPSC closely resembled wild-type organoids, exhibiting a mix of rod and cone photoreceptors, Müller, and amacrine cells after 170 days of differentiation and maturation. However, transcriptome analysis revealed differential splicing of neural and retinal disease-associated genes and changes in circular RNA expression, with only minor consequences on differential gene expression. These findings suggest that disturbances in gene splicing and circular RNA expression contribute to the RP phenotype.

P05

-Title-

Filip Karásek[1], David Staněk[1]

[1]*Institute of Molecular Genetics, CAS, Prague*

The biogenesis of new snRNP particles is complex regulated process that starts by transcription of snRNA. There are 5 major snRNAs, of which U1, U2, U4, U5 are transcribed by RNA polymerase II. Already during transcription the 5‘ end is modified to form 7-methylguanosine cap. This cap is recognized by series of proteins which are involved in snRNA export to cytoplasm. After export from nucleus, the heptamer of Sm protein, also called the Sm ring, is loaded onto snRNA. After Sm ring formation, the hypermethylation of the cap occurs to form 2,2,7-trimethylguanosine cap. Newly formed snRNP is reimported back to nucleus, where maturation of snRNPs continues in membraneless structures called Cajal bodies. The aim of my project is to describe mechanisms which are involved in quality control of snRNP during their biogenesis. Our data indicate different level of cap methylation between wild type and truncated U4 snRNA. I will investigate whether this phenomenon is general for other major snRNAs. Next, I plan to identify factors that discriminates between WT and truncated snRNAs and the role the 5' cap plays in snRNA biogenesis and quality control. I will downregulate TGS1, FTO and other enzymes that are involved in (de)methylation of the 5' end nucleotides to test whether these changes affect proofreading activity of snRNA biogenesis.

P06

tRNA synthetases as potential RNA capping enzymes

Jan Říha[1], Hana Cahová[1]

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

In our laboratory, we have discovered a novel type of 5' RNA caps in bacteria known as dinucleoside polyphosphate (NpnNs) caps. However, the role of these caps in cellular processes remains unclear and requires further investigation. To unravel their function, it is essential to understand their formation mechanism. One potential mechanism involves the incorporation of dinucleoside polyphosphates into RNA during transcription as non-canonical initiation nucleotides (NCINs). This mechanism has been demonstrated in vitro. Nevertheless, some NpnNs attached to RNA have not been previously detected in the free form in cells, suggesting the existence of an RNA capping enzyme. A compelling hypothesis is that tRNA synthetases could also function as 5' RNA capping enzymes in bacteria. These enzymes are excellent candidates due to their capability to produce free diadenosine polyphosphates. Instead of using ATP as a substrate for Ap4A formation, tRNA synthetases might accept triphosphate RNA, leading to the formation of Ap4A RNA caps. In this research project, we aim to explore the potential of tRNA synthetases as 5' RNA capping enzymes. To investigate this hypothesis, we selected and subcloned five tRNA synthetases from an E. coli cDNA library and expressed them in *E. coli*. His-tagged tRNA synthetases were then purified using immobilized metal affinity chromatography (IMAC, HisTrap), followed by size exclusion chromatography (SEC). Subsequently, we examined the production of free Ap4A using high-performance liquid chromatography (HPLC). Additionally, we will assess the capping potential of tRNA synthetases by conducting experiments with radioactively labelled RNA, followed by analysis on gel electrophoresis.

P07

Different mechanisms of stress granules dissolution

Jana Vojtová[1], Kristína Jendruchová[1], Tomáš Groušl[2], and Leoš Valášek[1]

[1]Laboratory of Regulation of Gene Expression,
[2]Laboratory of Cell Signalling, Institute of Microbiology of the Czech Academy of Sciences, 142 20, Praha 4, Czech Republic

Stress granules (SGs) are highly dynamic organelles that are formed within minutes of acute stress and dissolved within 1-2 hours after the stress release. While the temporary presence of the SGs is considered to be cytoprotective, the permanent presence of the SGs in the cell might be associated with the formation of pathological protein aggregates, which are one of the hallmarks of neurodegenerative diseases. We plan to focus on the dissolution of the SGs induced by heat shock in the model organism yeast Saccharomyces cerevisiae, which shows a high level of homology with the human genome and the presence of essential pathways relevant to human neurodegenerative diseases. We will compare a wild-type yeast strain to a strain with the limited dissolution of SGs, e.g., Hsp104 disaggregase deletion mutant (hsp104Δ). Our preliminary results indicate that there are several different pathways dedicated for the SGs clearance depending on whether SGs are formed temporary or are persistent. The dissolution of physiological temporary formed SGs might be promoted by translation initiation, while persistent SGs in hsp104Δ seemed to be cleared by autophagy. The obtained results will help us to better understand the possible pathological effect of the defective SGs dissolution on the cells.

P08

Computer simulations of the bacterial ribosome using a coarse-grained model

Josef Cikhart[*1], Aneta Leskourová, Michal H. Kolář

[1]*Department of Physical Chemistry, University of Chemistry and Technology, Prague*

Molecular dynamics simulations are a valuable tool for studying the conformational behavior of biomolecules and biomolecular complexes, such as the ribosome. However, one significant challenge with the ribosome is its size, making the execution of all-atom simulations for these systems highly demanding. To address this issue, one approach is to employ coarse-graining, which involves replacing groups of atoms with larger particles known as “beads.” This reduction in the system's degrees of freedom allows for longer simulation times, enabling the study of slower biomolecular processes compared to atomistic simulations. In this study, we aimed to gain a deeper understanding of the simulation parameters and performance of the coarse-grained (CG) model applied to the bacterial ribosome. Hence, we conducted a series of simulations and compared the results to atomistic simulations and experimental models. Specifically, we focused on the so-called “elastic networks,” which constitute a crucial component of the CG model responsible for maintaining the tertiary structure of the simulated system. Our findings indicate that CG simulations yield reasonable ribosome structures, regardless of the specific details of the elastic networks. However, the conformational dynamics of various ribosome components are notably influenced by the elastic network. These results not only establish a robust workflow for our future ribosome studies but also shed light on the specific questions about the translation machinery that can be effectively addressed through CG simulations.

P09

How flexibility of delta affects transcription in G+ bacteria

Klára Mikesková[1], Hana Šanderová, Libor Krásný

[1]*Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*

The δ subunit of bacterial RNA polymerase (RNAP) in Gram-positive bacteria consists of a structured N-terminal domain (NTD) separated from the intrinsically disordered, highly acidic C-terminal domain (CTD) by a positively charged lysine stretch (K-tract). Despite extensive studies of this protein, interactions of its CTD with RNAP and their role in transcription are virtually unknown. This project addresses this key aspect of the transcription machinery in Gram-positive bacteria, focusing on *Bacillus subtilis* and *Staphyloccocus aureus* δ subunits. The amino acid (aa) sequence homology between δ subunits from these two organisms is 64 %. Differences between these proteins include the absence of the K-tract from the *S. aureus* and markedly different aa sequences of their δ-CTDs. We created a panel of chimeric δ proteins from the two organisms with all combinations of NTD, K-tract, and CTD. We then performed *in vitro* transcription experiments with *B. subtilis* RNAP to assess the effects of these domains on the RNAP function. The experiments revealed that *S. aureus* δ does not complement *B. subtilis* δ on RNAP from *B. subtilis*. Furthermore, and most interestingly, CTD from *S. aureus* was unable to substitute CTD from *B. subtilis*, suggesting that the CTDs, even though they are both unstructured and highly acidic, are not interchangeable. These results provide a basis for future comparative studies of these intrinsically disordered protein domains.

P10

Downregulation of eIF3d and eIF3e increases expression of ribosomal proteins

Anna Herrmannová[1#], Jan Jelínek[2#], Klára Pospíšilová[1], Farkas Kerényi[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

A prominent hallmark of cancer is deregulated protein synthesis. Recent studies suggest that the unbalanced expression of eIFs is not only an indirect consequence of neoplasia but itself contributes significantly to cell transformation, tumor development, cancer cell survival and metastasis. Among them, eIF3 stands out as the largest complex composed of 12 subunits with a modular assembly, where aberrant expression of one subunit generates only partially functional subcomplexes. Here, we took advantage of well-established knock-downs of subunits d, e and h of human eIF3 in HeLa cells and investigated their impact on differential gene expression translatome-wide by Ribo-Seq. We demonstrated that depletion of eIF3e and partially eIF3d increases the expression of a number of ribosomal proteins, implicating eIF3 in controlling the balanced production of mature ribosomes. Together, our data illustrate that individual eIF3 subunits exert specific translational control over a broad range of cellular transcript

P11

Substrate Prediction Model of Human NudiX Enzymes

Klára Viktorinová[1], Martin Lepšík[1], Ondřej Nešuta[1] and Hana Cahová[1]

[1]*Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic*

NudiX enzymes are known for their ability to hydrolyse a polyphosphate backbone attached to a nucleoside. They are present in viruses, prokaryotic as well as eukaryotic organisms, where they are responsible for cleavage of various nucleotides and other small molecules containing phosphate. They are also known to cleave stress sensing alarmones – dinucleoside polyphosphates (NpnNs). In addition to canonical 7-methylguanosine, NpnNs also have been reported to form 5´ RNA caps. Resulting NpnN-RNA can be as well substrate for NudiX enzymes. The human genome encodes 24 enzymes from NudiX superfamily with miscellaneous substrate preference. However, only some of the human NudiXes have been purified and studied against limited number of substrates. Since the substrate specificity of many human NudiXes is still unclear, their role in human cells cannot be fully explained. In the initial part of our study, we focused on purification and characterisation of various human NudiXes, including HsNudt2, HsNudt5, HsNudt9 and HsNudt12. First, we tested them against NpnNs, following the hydrolysis using high-performance liquid chromatography (HPLC). Second, we prepared NpnN-RNAs by in vitro transcription and quantified RNA decapping effect of the NudiXes using polyacrylamide gel electrophoresis. Later, the information about substrate specificity of studied NudiXes will be used in computer modelling of the substrates into enzyme structures. The resulting substrate binding prediction model could be applied on yet unexplored NudiX enzymes.

P12

Differentiation of hiPSC into RPE cells and Characterisation of Splicing Factor Mutants

Luisa Howainsky[1,2], Felix Zimmann[2], David Staněk[2]

[1]Hochschule Zittau/Görlitz, Fakulty Natural and Environmental Sciences, Theodor-Körner-Allee 16, 02763 Zittau, Germany
[2]Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of RNA Biology, Vídeňská 1083, 142 200 Prague 4, Czech Republic

Retinitis pigmentosa (RP) is a heterogeneous subset of inherited retinopathies characterised by progressive loss of photoreceptor and retinal pigment epithelium (RPE) cells, eventually leading to blindness. Mutations within pre-mRNA splicing factors have been linked to the occurrence of RP. Among those is the pre-mRNA processing splicing factor 8 (PRPF8), which is a core protein of the spliceosome. Human induced pluripotent stem cell (hiPSC) derived RPE cells represent a promising model for studying PRPF8-related RP since animal models have fallen short of mimicking the human RP phenotype. This project focuses on the optimisation and comparison of two previously described protocols for the differentiation of hiPSC into RPE. The Y2334N mutation in the C-terminus of the PRPF8 gene, which is associated with RP pathogenesis, was introduced to the hiPSC. RT-PCR expression analysis of eye field and RPE markers during differentiation of hiPSC, using the rapid-directed and spontaneous approach, has proven the ability of Y2334N-mutated hiPSC to differentiate towards RPE. However, the expression of RPE-specific marker RPE65 exhibited an upregulation at an earlier time point when using the directed differentiation, implying an enhanced efficiency for this protocol. RPE-like cells were cultivated on Transwell® inserts to study the effect of the Y2334N mutation on RPE integrity by transepithelial electrical resistance (TEER) measurement. Furthermore, growth factor secretion was analysed using ELISA. However, optimisation of sample collection and confluency of cell layers appeared to be crucial for constant results for both methods - an aspect, which entails potential for future investigations.

P13

Exploring the Evolutionary Stability of the Peptidyl Transferase Center in Ribosomes using Computer Simulations

Martin Mašek*[1], Michal H. Kolář

[1]*Department of Physical Chemistry, University of Chemistry and Technology, Prague*

The ability of ribosomes to catalyze peptide bond formation is attributed to the peptidyl transferase center (PTC), a catalytic site composed solely of ribosomal RNA (rRNA). Within the ribosome, a structurally symmetric region envelops the PTC, and this feature has been observed consistently across diverse species. This intriguing symmetry hints at an ancient evolutionary origin, suggesting that over billions of years, the PTC has evolved through the accretion of rRNA and ribosomal proteins. However, the precise roles of ribosomal proteins (rProteins) and rRNA in conferring stability to the PTC remain elusive, with the prevailing assumption that the PTC has become less flexible over time. To explore this hypothesis, we conducted all-atom molecular dynamics simulations on two ancestral ribosomes, reconstructed from a modern ribosomal template. We focused on PTC constructs incorporating various rProtein fragments. The simulations reveal striking structural similarities between ancestral and modern PTCs. Furthermore, the interactions between rProteins and rRNA appear to play a crucial role in maintaining the conformational stability of the PTC, potentially enhancing the specificity of peptide bond formation. Our findings provide valuable insights into the molecular mechanisms underpinning the evolutionary stability of the PTC within ribosomes.

P14

The role of coilin in snRNP biogenesis

Nenad Radivojević[1], David Staněk[1]

[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of RNA Biology, Vídeňská 1083, 142 200 Prague 4, Czech Republic

Cajal bodies (CB) are nuclear non-membrane formations involved in the metabolism of spliceosomal small nuclear ribonucleoprotein particles (snRNPs). During their biogenesis snRNPs have been observed to transiently localize to CB, while disruption of their maturation leads to their accumulation. While their implication in snRNP maturation is evident, the precise mechanism and function remain elusive. Coilin, a CB marker protein shown to provide scaffolding and induce CB formation, possibly serves as a targeting signal and a 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 various classes of RNA. In this study, we aim to uncover the nature of the interaction between coilin and snRNPs and determine whether coilin can differentiate between mature and immature snRNP particles. Here, we identify RG-box as an RNA-binding domain able to pull down snRNA in vitro. Notably, arginines within the RG box provide the interaction interface, potentially mediated by electrostatic interactions. Using C214 coilin fragment and several of its mutated variants, we were able to show that both the RG-box and the Tudor-like domain are necessary for the efficient association with U2 snRNP. In future, we plan to solve the structure of C214 fragment in complex with the maturing snRNP, and elucidate the role of coilin as a quality control factor in snRNP assembly.

P15

Diadenosine tetraphosphate (Ap_4A) serves as a 5' RNA cap in mammalian cells

Jiří František Potužník[1, 2, 3], Ondřej Nešuta[1, 3], Anton Škríba[1], Barbora Voleníková[1], Maria-Bianca Mititelu[1, 2], Flaminia Mancini[1, 2], Valentina Serianni[1, 2], Henri Fernandez[1], Kristína Spustová[1], Jana Trylčová[1], Pavel Vopalensky[1] and Hana Cahová[1]

[1]Chemical Biology of Nucleic Acids, Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czechia
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czechia
[3]These authors contributed equally to this work.

The recent expansion of the field of RNA chemical modifications has changed our understanding of post-transcriptional gene regulation. Apart from internal nucleobase modifications, 7-methylguanosine was long thought to be the only eukaryotic RNA cap. However, the discovery of non-canonical RNA caps in eukaryotes revealed a new niche of previously undetected RNA chemical modifications. We are the first to report the existence of a new non-canonical RNA cap - diadenosine tetraphosphate (Ap_4A) - in human and rat cell lines. Ap_4A is the most abundant dinucleoside polyphosphate in eukaryotic cells and can be incorporated into RNA by RNA polymerases as a non-canonical initiating nucleotide (NCIN). Using liquid chromatography-mass spectrometry (LC-MS), we show that the amount of capped Ap_4A-RNA is independent of the cellular concentration of Ap_4A. A decapping enzyme screen revealed identifies two enzymes cleaving Ap_4A-RNA - NUDT2 and DXO, both of which also cleave other substrate RNAs *in vitro*. We further assess the translatability and immunogenicity of Ap_4A-RNA and show that although it is not translated, Ap_4A-RNA is recognized as self by the cell and does not elicit an immune response, making it a natural component of the transcriptome. Our finding opens a previously unexplored area of eukaryotic RNA regulation.

P16

Correcting splicing of Prpf31 in retinitis pigmentosa - A step towards finding a cure

Poulami Banik[1] and David Staněk[1]

[1]Laboratory of RNA biology, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic

Retinitis pigmentosa(RP) is a genetic disorder causing the loss of photoreceptors and consequently central and peripheral vision. Pre-mRNA processing factor 31 (Prpf31) is a splicing factor associated with ~10% of cases of the autosomal dominant form of RP. We have described a novel point mutation in the intron 10 of the PRPF31 gene which caused RP in one of the siblings, while the parents were phenotypically unaffected. The splicing efficiency test of intron 10 using human cell lines and blood samples showed that intron 10 is not efficiently removed even in wild-type samples. Next, we established a splicing reporter based on PRPF31 intron 10 and inserted the intronic mutation. The mutation abolished the splicing of intron 10. Then, we designed antisense oligonucleotides (ASOs) targeting the potential binding sites of splicing regulators in exons 10 and 11 and intron 10. We introduced ASOs individually or in combination into cells and evaluated their effect on the splicing of wild-type and mutated Prpf31. ASOs are becoming a powerful tool to manipulate RNA processing and hold a therapeutic potential to treat various genetic disorders. Here, we identified ASOs to improve the splicing of PRPF31, one of the most mutated genes causing RP, which may open a way to treat this rare disease.

P17

Assembly factor mtSAF24 in the biogenesis of small mitoribosomal subunit in trypanosomes

Prashant Chauhan[1, 2], Alena Zíková[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*

Mitoribosomes are responsible for synthesis of proteins encoded in mitochondrial genomes. Biogenetic pathway of small mitoribosomal subunit (mtSSU) in parasitic protist, *Trypanosoma brucei* includes four structurally characterized precursors. The earliest precursor, known as assemblosome (~4MDa), contains immature 9S rRNA complexed with an incomplete set of mtSSU proteins and 34 assembly factors (AFs). The assemblosome features a protrusion on the immature intersubunit side, capped with a low-resolution disc-shaped structure of unknown composition, possibly representing a piece of the inner mitochondrial membrane (IMM). The structure contacts solely the N-terminal domain of the AF termed mtSAF24. Mitochondrial fractionation showed that mtSAF24 and other AFs of the assemblosome are predominantly present in the peripheral membrane fraction, indicating that the assemblosome is associated with the IMM. Ablation of mtSAF24 by RNA interference (RNAi) or knockout by CRISPR-Cas9 results in strong growth defects accompanied by the loss of mtSSU rRNA, documenting the essential role of mtSAF24 in mtSSU assembly. Unexpectedly, the expression of CTD of mtSAF24 rescued the growth phenotype completely, indicating that the linkage between the assemblosome and the peripheral disc is not essential. Furthermore, the assemblosome without the NTD of mtSAF24, as well as two later precursors, which lack mtSAF24 completely, localized to the membrane fractions. These results indicate that mtSSU assembly in *T. brucei* occurs in the association with IMM, which has not been reported in any organism. However, Contrary to our initial hypothesis, the binding to IMM is not mediated only by mtSAF24.

P18

Rifampicin: stops or stimulates transcription ?

Tamara Balgová[1], Petra Sudzinová[1], Dragana Vítovská[1], Křeňková Alena[2], Hubálek Martin[2] and Libor Krásný[1]

[1]*Institute of Microbiology, The Czech Academy of Sciences, Czech Republic*
[2]*Institute of Organic Chemistry and Biochemistry, The Czech Academy of Sciences Czech Republic*

Nowadays, antibiotic resistance has become a serious worldwide problem. One of the clinically important antibiotics is rifampicin, a key component of anti-tuberculosis therapy. Rifampicin binds to RNA polymerase (RNAP) and stops the synthesis of RNA at an early phase of transcription. Protein HelD is an interaction partner of RNAP, dissociating stalled complexes RNAP from DNA, thereby enabling transcription restart. At present, three different classes of HelD proteins are known (I-III). So far, only Class II HelD proteins (found in *e. g. Actinobacteria*) were shown to provide resistance against rifampicin; Class I HelD proteins (*Firmicutes*) were suggested to lack this property. Here, we investigated whether *Bacillus subtilis* HelD might play a role in rifampicin resistance. Contrary to published results, we showed that the presence of the *helD* gene increased the minimal inhibitory concentration (MIC) to rifampicin. Next, we demonstrated that rifampicin induces expression of the *helD* gene. Finally, we identified the 5' untranslated region of the *helD* gene is responsible for this induction, and showed that the regulation is at the transcriptional and not translational level. 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*

P19

Reactivation of RNA interference pathway in mammals

Valeria Buccheri[1], Radek Malik[1], Filip Horvat[1,2], Eliska Taborska[1], Josef Pasulka[1], Petr Svoboda[1].

[1]Laboratory of Epigenetic Regulations, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic.
[2]Bioinformatics Group, Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia.

RNA interference (RNAi) is a sequence-specific gene silencing mechanism initiated by Dicer, which cleaves long double-stranded RNA molecules into ~22 nucleotides-long short interfering RNAs (siRNAs). RNAi acts as an antiviral innate immunity and genome defense system in plants and invertebrates, whereas its canonical roles in mammals are only restricted to mouse oocytes; this is due to the expression of a short Dicer isoform denoted DicerO, which lacks the N-terminal helicase domain and is adapted to siRNAs production. In order to understand why RNAi is only active in mouse oocytes but remains marginal in somatic cells, and whether it could be restored in vivo, we generated a genetically modified mouse model where the full-length Dicer was replaced with a truncated Dicer isoform (DicerΔHEL1), which mimics DicerO. In addition to DicerΔHEL1 variant, this mouse model carries a transgene acting as long dsRNAs source, and a reporter gene that acts as RNAi target and allows us to monitor RNAi activity. Here we report phenotype characterizations of our DicerΔHEL1 mouse model with focus on its ability to process long dsRNAs into siRNAs and the effect of those siRNAs on the target gene. Interestingly, heart and skeletal muscle show the highest RNAi activity in vivo under the above-mentioned conditions.

P20

Novel transcription factors in *Bacillus subtilis*

Veronika Kočárková[1], Hana Šanderová, Jiří Pospíšil and Libor Krásný

[1]*Institute of Microbiology of the CAS, Prague, Czech Republic*

Bacteria have evolved a broad range of mechanisms to regulate gene expression, allowing them to adapt to changing environments. A fundamental role in this regulation play transcription factors (TFs). An important group of TFs binds to regulatory regions of DNA and either prevents or promotes RNA polymerase association with promoter DNA. Many of these TFs remain unknown. *Bacillus subtilis*, despite being one of the best-characterized model organisms, contains more than 30 % of genes with unknown functions. Using a deep-learning approach, putative TFs were predicted in B. subtilis and other organisms. From the list of predicted TFs, we selected 15 top candidates containing the helix-turn-helix motif, known as the most widely used DNA-binding motif in the prokaryotic world. This project aims to unravel the functions of the 15 selected uncharacterized putative TFs. To get first insights into the cellular roles of these proteins, deletion strains were prepared and evaluated in phenotypic assays followed by proteomic and transcriptomic analyses. We also prepared strains with FLAG-tagged versions of these TFs for ChIPseq experiments to identify their binding sites in the genome. Additionally, immunoprecipitation of these FLAG-tagged TFs will be performed to identify their interacting partners. Finally, the 3D structures of these proteins will be determined. Taken together, this project will provide a better understanding of how an organism responds to varying environmental conditions through transcriptional regulation.

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

P21

Stops making sense - lessons from the tRNA anticodon stem

Zuzana Pavlikova[1], Petra Miletinova[1], and Leoš Shivaya Valášek[1]

[1]*Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic*

Imagine a gene is a sentence starting with a capital letter and ending with a full stop, and a genome is a book telling an entire story. In some protozoan organisms, extra full stops have infiltrated the sentences, replacing specific letters of arbitrary words (namely E and W). As a r.sult, readers (in this cas. ribosomes) are confus.d as to .here thes. sentences really .nd and th. story becom.s disjointed. .e describ.d a molecular mechanism that thes. organisms must hav. developed to allo. the reader to navigat. the s.ntences as if there .ere no .xtra full stops. Th. sentences are so specifically .ncrypted that read.rs of no other organisms but thos. very fe. can decipher the story in a prop.r .ay. The trick lies in the l.ngth of the transf.r RNA molecul., and in the uniqu. modification of a singl. protein that normally ensur.s dot recognition in c.lls — the precis. end of prot.in synthesis. By the interaction of these two modified molecules, the ribosome of this protozoan knows when to correctly terminate, notwithstanding the many dots, and when to replace the dots with the two original letters (E and W) by the process called stop codon readthrough, which returns meaning to the genetic information. Furthermore I will demonstrate that these so-called readthrough inducing tRNAs establish critical contacts between their anticodon stem and small ribosomal proteins forming the decoding site, which markedly increase the probability of their selection during the codon sampling and accommodation stages of elongation.

P22

Downregulation of eIF3d and eIF3e decreases expression of key components of the activated MAPK/ERK signaling pathway

Anna Herrmannová[1], Jan Jelínek[2], Klára Pospíšilová[1], Farkas Kerényi[1], Tomáš Vomastek[3], Mahabub Pasha Mohammad[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
[3]Laboratory of Cell Signaling, Institute of Microbiology of the Czech Academy of Sciences, Prague, Vídeňská 1083, 142 20, Czech Republic

A prominent hallmark of cancer is deregulated protein synthesis. Recent studies suggest that the unbalanced expression of eIFs is not only an indirect consequence of neoplasia but itself contributes significantly to cell transformation, tumor development, cancer cell survival and metastasis. Among them, eIF3 stands out as the largest complex composed of 12 subunits with a modular assembly, where aberrant expression of one subunit generates only partially functional subcomplexes. Here, we took advantage of well-established knock-downs of subunits d, e and h of human eIF3 in HeLa cells and investigated their impact on differential gene expression translatome-wide by Ribo-Seq.
We demonstrate that knock-downs of eIF3d and eIF3e lead indirectly to activation of the MAPK/ERK1/2 signaling pathway, which is frequently upregulated in cancer, despite overall reduced protein levels of its multiple components. Together, our data illustrate that individual eIF3 subunits exert specific translational control over a broad range of cellular transcripts.

LIST OF PARTICIPANTS

Vojtěch Andrle
East Port Praha
vojtech.andrle@eastport.cz

Tamara Balgová
Institute of Microbiology, CAS, Prague
tamara.balgova@biomed.cas.cz

Poulami Banik
Institute of Molecular Genetics, CAS, Prague
poulami.banik@img.cas.cz

Robert Beal
Bio-Rad Laboratories
robert_beal@bio-rad.com

Filip Brázdovič
Institute of Microbiology, CAS, Prague
filipbrazdovic@gmail.com

Valeria Buccheri
Institute of Molecular Genetics, CAS, Prague
valeria.buccheri@img.cas.cz

Hana Cahová
Institute of Organic Chemistry and Biochemistry, CAS, Prague
cahova@uochb.cas.cz

Prashant Chauhan
Institute of Parasitology, Biology Centre, CAS, České Budějovice
pra751990@gmail.com

Nathalia B. Chitiva
Institute of Parasitology, Biology Centre, CAS, České Budějovice
nathalia.ballesteros@paru.cas.cz

Jana Chumová
Institute of Microbiology, CAS, Prague
jana.chumova@biomed.cas.cz

Josef Cikhart
Department of Physical Chemistry, University of Chemistry and Technology, Prague
cikhartj@vscht.cz

Alžbeta Dikunová
CEITEC, Masarky University, Brno
alzbeta.dikunova@ceitec.muni.cz

David Doležel
Institute of Entomology, Biology Centre, CAS, České Budějovice
david.dolezel@entu.cas.cz

Ondřej Gahura
Institute of Parasitology, Biology Centre, CAS, České Budějovice
gahura@paru.cas.cz

Sebastian Glatt
Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland
sebastian.glatt@uj.edu.pl

Ivana Hálová
Institute of Molecular Genetics, CAS, Prague
ivana.halova@img.cas.cz

Jana Heřmanská
Trigon Plus, s.r.o.
hermanska@trigonplus.cz

Anna Herrmannová
Institute of Microbiology, CAS, Prague
herrmannova@seznam.cz

Kamila Horáčková
Faculty of Science, Charles University, Prague
horackovakamila166@seznam.cz

Luisa Howainsky
Institute of Molecular Genetics, CAS, Prague
luisa.howainsky@gmail.com

Aneta Hrádková
Department of Physical Chemistry, University of Chemistry and Technology, Prague
hradkova@vscht.cz

Pavlína Jelínková
Bio-Rad Laboratories
pavlina_jelinkova@bio-rad.com

Kristína Jendruchová
Institute of Microbiology, CAS, Prague
kristina.jendruchova@biomed.cas.cz

Filip Karásek
Institute of Molecular Genetics, CAS, Prague
karasekf@outlook.cz

Liam P. Keegan
CEITEC, Masarky University, Brno
liam.keegan@ceitec.muni.cz

Veronika Kočárková
Institute of Microbiology, CAS, Prague
veronika.kocarkova@biomed.cas.cz

Michal Kolář
Department of Physical Chemistry, University of Chemistry and Technology, Prague
Michal1.Kolar@vscht.cz

Jiří Koubek
ZMBH, Heidelberg University, Germany
j.koubek@zmbh.uni-heidelberg.de

Tracy Chih-Ting Koubková Yu
COS, Heidelberg University, Germany
tracyyu770213@gmail.com

Julie Kovářová
Institute of Parasitology, Biology Centre, CAS, České Budějovic
julie.kovarova@paru.cas.cz

Natalia Kraeva
Life Science Research Centre, University of Ostrava
luzikhina@gmail.com

Zuzana Kráľová
Institute of Organic Chemistry and Biochemistry, CAS, Prague
zuzana.kralova@uochb.cas.cz

Adam Krayzel
Life Science Research Centre, University of Ostrava
adamkrayzel@gmail.com

Vojtěch Ledvina
East Port Praha
vojtech.ledvina@eastport.cz

Martin Mašek
Department of Physical Chemistry, University of Chemistry and Technology, Prague
martin.masek19@gmail.com

Klára Mikesková
Institute of Microbiology, CAS, Prague
klara.mikeskova@biomed.cas.cz

Matěj Miláček
Institute of Entomology, Biology Centre, CAS, České Budějovice
mmilacek02@gmail.com

Petra Miletínová
Institute of Microbiology, CAS, Prague
miletinovapetra@gmail.com

Pavla Musilova
CEITEC, Masarky University, Brno
pavla.musilova@ceitec.muni.cz

Ondrej Nešuta
Institute of Organic Chemistry and Biochemistry, CAS, Prague
nesuta@uochb.cas.cz

Nikola Nosková
CEITEC, Masarky University, Brno
noskova.16@gmail.com

Mary O'Connell
CEITEC, Masarky University, Brno
mary.oconnell@ceitec.muni.cz

Zdeněk Paris
Institute of Parasitology, Biology Centre, CAS, České Budějovice
parda@paru.cas.cz

Josef Pasulka
Institute of Molecular Genetics, CAS, Prague
josef.pasulka@img.cas.cz

Zuzana Poláčková
Institute of Molecular Genetics, CAS, Prague
zuzana.cihlarova@img.cas.cz

Martin Pospíšek
Faculty of Science, Charles University, Prague
martin@natur.cuni.cz

Klára Pospíšilová
Institute of Microbiology, CAS, Prague
klara.pospisilova@biomed.cas.cz

Nenad Radivojević
Institute of Molecular Genetics, CAS, Prague
nenad.radivojevic@img.cas.cz

Jan Říha
Institute of Organic Chemistry and Biochemistry, CAS, Prague
jan.riha@uochb.cas.cz

Adriana Roithová
Institute of Microbiology, CAS, Prague
adriana.roithova@biomed.cas.cz

Kristina Roučová
Faculty of Science, Charles University, Prague
kristina.roucova@natur.cuni.cz

Samanta Rožánková
Institute of Organic Chemistry and Biochemistry, CAS, Prague
samanta.rozankova@uochb.cas.cz

Tania Sanchez Quirante
Institute of Organic Chemistry and Biochemistry, CAS, Prague
tania.sanchez@uochb.cas.cz

Marek Šebesta
CEITEC, Masarky University, Brno
marek.sebesta@ceitec.muni.cz

Anna Smirnova
Institute of Microbiology, CAS, Prague
anna.smirnova@biomed.cas.cz

Vlastimil Smýkal
Institute of Entomology, Biology Centre, CAS, České Budějovice
v.smykal@entu.cas.cz

Richard Štefl
CEITEC, Masarky University, Brno
richard.stefl@ceitec.muni.cz

Adriana Šubrtová
Institute of Microbiology, CAS, Prague
adriana.subrtova@biomed.cas.cz

Petr Svoboda
Institute of Molecular Genetics, CAS, Prague
svobodap@img.cas.cz

Prasoon Kumar Thakur
Institute of Molecular Genetics, CAS, Prague
prasoont@img.cas.cz

Matouš Tulpa
Institute of Organic Chemistry and Biochemistry, CAS, Prague
matous.tulpa@uochb.cas.cz

Leoš Valášek
Institute of Microbiology, CAS, Prague
valasekl@biomed.cas.cz

Branislav Večerek
Institute of Microbiology, CAS, Prague
vecerek@biomed.cas.cz

Klára Viktorinová
Institute of Organic Chemistry and Biochemistry, CAS, Prague
klara.viktorinova@uochb.cas.cz

Jana Vojtová
Institute of Microbiology, CAS, Prague
jana.vojtova@biomed.cas.cz

Martin Volek
Institute of Organic Chemistry and Biochemistry, CAS, Prague
martin.volek@uochb.cas.cz

Pavel Vopálenský
Institute of Organic Chemistry and Biochemistry, CAS, Prague
pavel.vopalensky@centrum.cz

Dragana Vukič
CEITEC, Masarky University, Brno
dragana.vukic@ceitec.muni.cz

Richard Zelenka
MGP s.r.o.,Zlín
richard.zelenka@mgp.cz

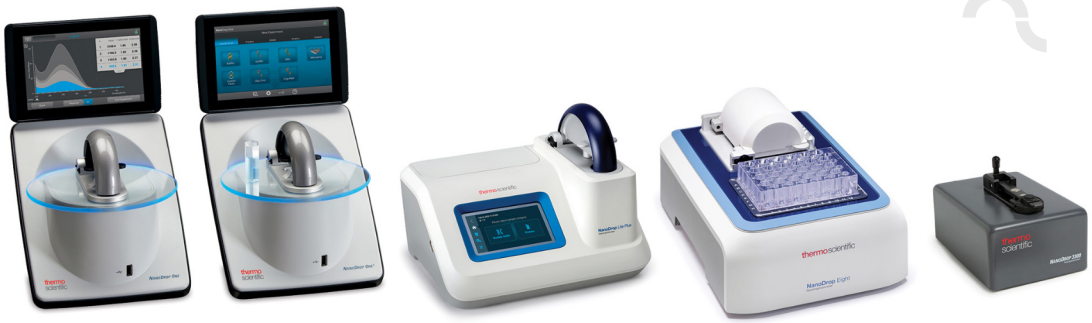
Felix Zimmann
Institute of Molecular Genetics, CAS, Prague
felix.zimmann@img.cas.cz

Jsme dodavatelem ND family:

NanoDrop produkty od Thermo Scientific

Přístroje na rychlou analýzu vzorků DNA, RNA a proteinů v mikroobjemech

Poskytujeme jako jediní v ČR autorizovaný servis k těmto přístrojům.



M.G.P. spol. s r. o., Kvítková 1575, Zlín 760 01, Česká republika
www.mgp.cz

RNA CLUB 2023

15 % sleva pro účastníky

Promo kód: **RNACLUB2023**

Sleva se vztahuje pouze na nově objednávané zboží a nezahrnuje přístrojovou techniku. Platnost akce je do **31. 11. 2023**.

RNasin® Plus Ribonuclease Inhibitor *kat. č.* **N2515**

Chcete ochránit svojí RNA před všudypřítomnými RNázami? Není lepší ochrany než léty prověřený RNasin od Promegy, který inhibuje široké spektrum RNáz bez toho, aniž by inhiboval jakékoli enzymy používané při PCR. Navíc je RNasin Plus stabilní i při 70 °C, takže je možné ho přidat do směsi před RT-PCR a zvýšit tak ochranu.

GoScript™ reverní transkriptáza *kat. č.* **A5001**

Novější a dokonalejší verze M-MLV transkriptázy se zesílenými funkcemi. Zaručuje vysokou odolnost vůči inhibitorům, spolehlivě přepisuje dlouhé i krátké transkripty včetně sekundární struktury. Navíc poskytuje citlivou transkripci low-copy i high-copy cílů.

M-MLV revezní transkriptáza *kat. č.* **M1705**

Léty a mnoha uživateli vyzkoušený standard. Skvělý poměr cena/výkon.

RiboPOOLs *kat. č.* **riboPOOL**

Vysoce účinná a efektivní deplece rRNA. Jedná se o pooly, které jsou složené z biotinylovaných DNA sond speciálně navržených pro konkrétní organismus, druh a buněčnou linii.

EASTPORT LIFESCIENCE

East Port Praha s.r.o., Možného 1065/10, 161 00 Praha 6, Česká republika
www.eastport.cz eastport@eastport.cz 800 100 529 235 318 177

Izolátory nukleových kyselin Maxwell nově i s IVDR

Maxwell jsou kompaktní automatizované platformy pro purifikaci nukleových kyselin, které zpracovávají současně až 16 (Maxwell® CSC) nebo až 48 vzorků (Maxwell® CSC 48). Použitím předplněných kazet přináší konzistentní a spolehlivou purifikaci DNA nebo RNA z různých typů vzorků. Intuitivní grafické rozhraní usnadňuje používání přístroje.

Aktuálně máme k dispozici tyto IVDR kity:

- Maxwell® CSC RNA Blood Kit
- Maxwell® CSC DNA Blood Kit
- Maxwell® CSC DNA FFPE Kit
- Maxwell® CSC Genomic DNA Kit
- Maxwell® CSC RNA FFPE Kit
- Maxwell® CSC Viral Total Nucleic Acid Kit
- Maxwell® CSC Whole Blood DNA Kit

Ukázka Maxwell předplněných kazet

Předností Maxwellu CSC a CSC 48 je mimo jiné **možnost přepínání v rámci IVD (diagnostického) a RUO (výzkumného) módu**. Díky tomu je možné pracovat s jedním přístrojem jak v režimu IVD (kity Maxwell CSC) tak v režimu RUO (ekonomicky výhodnější kity Maxwell RCS).
Technologicky přístroje vychází z izolátorů Maxwell RSC a Maxwell RSC 48 které i nadále zůstávají v nabídce pro akademické, univerzitní a veterinární laboratoře a výzkumné klinické aplikace.

Proč právě Maxwell® RSC?

Reference
Přístroje jsou umístěné v laboratořích s různým provozem a objemem vzorků. Naše izolátory najdete na odděleních mnoha fakultních nemocnic (Ostrava, Plzeň, Olomouc, Brno, Praha), soukromých diagnostických laboratoří i akademických a univerzitních pracovišť.

Aplikační flexibilita
Maxwell® RSC je uzavřený systém a k dispozici je široké spektrum kitů určených na konkrétní typy vstupních vzorků (krev, buňky, tělní tekutiny, tkáně, FFPE atd.). Tím však není omezeno použití pouze na tyto vzorky. K dispozici je celá řada aplikačních protokolů s optimalizací právě pro tyto nestandardní vzorky. Současně Promega neustále vyvíjí nové kity. Nové protokoly jsou pak zdarma k dispozici samostatně či v pravidelných ročních aktualizacích.

Spolehlivost
Jedena z prvních instalací Maxwellu byla v roce 2008 i přes kadodenní provoz v hemato-onkologické laboratoři je stále plně funkční, a to bez jediného servisního zákroku. Stejně jsou na tom další nainstalované Maxwelly u našich zákazníků.

Vyzkoušení u vás v laboratoři zdarma
Pokud vás koncept Maxwell® zaujal, neváhejte nás kontaktovat. Rádi vám poradíme, spojíme s referenčním zákazníkem, popřípadě zprostředkujeme podporu přímo od dodavatele. V případě že projevíte zájem, tak stále probíhá naše akce nezávazného vyzkoušení přístroje přímo ve vaší laboratoři zdarma.

EASTPORT LIFESCIENCE

East Port Praha s.r.o., Možného 1065/10, 161 00 Praha 6, Česká republika
www.eastport.cz eastport@eastport.cz 800 100 529 235 318 177

Produktové portfolio:

- Genomika
- Proteomika
- Chromatografie

GelDoc Go Imaging System

CFX Opus Real-Time PCR System

BIO-RAD

QX 600 ddPCR system

PTC Tempo Thermal Cycler

V případě dotazů, neváhejte kontaktovat obchodní zástupce společnosti Bio-Rad pro LSG divizi:

Ing. Pavlína Jelínková,Ph.D.: pavlina_jelinkova@bio-rad.com; mobil: +420 606 631 319

Ing. Jana Nováková: jana_novakova@bio-rad.com; mobil: +420 602 752 524

Bio-Rad spol. s.r.o.
Na Strži 1702/65
140 00 Praha 4
IČ: 49243764

Trigon plus®

...řešení pro vaši laboratoř

Laboratorní přístroje pro LifeScience, klinické, farma a biotechnologické laboratoře

Multiskan-Varioskan

univerzální mikrodestičkové spektrofotometry, absorbance, fluorescence, luminiscence měření malých objemů od 2 µl, klasických mikrodestiček i kyvet

Herasafe 2025

nový model unikátní řady biohazard-laminárních boxů pro ochranu obsluhy i produktu splňující nejpřísnější normy

ThermoFisher SCIENTIFIC

Heracell Vios 160i

inkubátory s řízenou atmosférou CO2 a N2 , kontinuální HEPA filtrace vnitřní atmosféry, individuální kultivační komůrky

Cell Locker™

Multifuge X1/X4 Pro

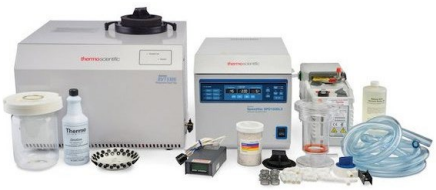
nová řada multifunkčních centrifug pro výzkumné účely i rutinu
unikátní nové funkce usnadňující obsluhu

Sorvall LYNX 4000/6000

velkoobjemová vysokootáčková centrifuga
kapacita až 6 000 ml
výkon až 100 605 x g
výkyvné i úhlové rotory

SpeedVac

vakuové centrifugační koncentrátory všech kapacit, pro všechny typy aplikací, lyofilizátory



ThermoFisher SCIENTIFIC

Thermo TDE -86°C

hlubokomrazicí boxy splňující vysoké nároky na bezpečnost vzorků i ekonomiku,
velká kapacita až 60000 x 2 ml

PhO2x Box

nový flexibilní koncept hypoxické kultivace, atmosféra CO2 i O2, několik variant komor pro až 18 lahví

Baker

www.trigonplus.cz