# POLISH RNA BIOLOGY MEETING

## September 28-30, 2023
## Warsaw, Poland

# Contents

# Welcome

*On the behalf of the Scientific Committee and the Organizing Committee from the International Institute of Molecular and Cell Biology in Warsaw (IIMCB) we would like to warmly welcome you on the* ***RNA Biology Meeting 2023 in Warsaw****.*

*RNA, once regarded as a mere intermediary in the flow of genetic information, has emerged as a pivotal player in regulating diverse biological processes, from gene expression to protein synthesis. Recent breakthroughs in RNA research have revolutionised medicine, ushering us in a new era of genome editing and mRNA vaccines. Despite remarkable progress, much remains to be uncovered about RNA's role in organismal physiology and pathology, as well as its potential in medical and industrial applications. Our conference brings together keynote speakers from around the world alongside a thriving and expanding community of Polish RNA researchers.*

*We hope that this event will serve as a platform for sharing findings and ideas, networking, nurturing existing and establishing new collaborations, and catalysing advancements in RNA biology.*

Prof. Gracjan Michlewski
Chair of the Scientific Committee

Prof. Andrzej Dziembowski
Chair of the Organizing Committee

# Committees & Organizer

## Scientific Committee

**Gracjan Michlewski – Chair**, IIMCB, Warsaw, Poland and University of Edinburgh, UK

**Sebastian Glatt**, Malopolska Centre of Biotechnology, Jagiellonian University in Kraków, Poland

**Jacek Jemielity**, University of Warsaw, Poland

**Kinga Kamieniarz-Gdula**, Adam Mickiewicz University, Poznań, Poland

**Elzbieta Kierzek**, Institute of Bioorganic Chemistry, PAS, Poznań, Poland

**Joanna Kufel**, University of Warsaw, Poland

## Organizing Committee

**Andrzej Dziembowski – Chair**, IIMCB, Poland

**Urszula Białek-Wyrzykowska**, IIMCB, Poland

**Kamila Gajdek**, IIMCB, Poland

**Daria Goś**, IIMCB, Poland

**Dorota Libiszowska**, IIMCB, Poland

**Marcin Ogonowski**, IIMCB, Poland

**Iwona Pilecka**, IIMCB, Poland

**Agnieszka Potęga**, IIMCB, Poland

**Eliza Ratkowska**, IIMCB, Poland

**Jakub Skaruz**, IIMCB, Poland

**Agata Szulim**, IIMCB, Poland

**Agnieszka Wilamowska**, IIMCB, Poland

## Organizer

**International Institute of Molecular and Cell Biology in Warsaw (IIMCB)**
4 Ks. Trojdena Street
02-109 Warsaw, Poland

The Polish RNA Biology Meeting is supported by the MOSaIC project which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 810425.

**The Polish RNA Biology Meeting**, supported by the European Union's Horizon 2020 research and innovation program ends the project entitled *"MOlecular Signaling in Health and Disease - Interdisciplinary Centre of Excellence"* (MOSaIC), which has been implemented at the IIMCB in years 2019-2023. MOSaIC has largely contributed to the Institute's strategic scientific and organizational developments. It supported the establishment of the Laboratory of RNA Biology - ERA Chairs Group led by Prof. Andrzej Dziembowski, which has strengthened the IIMCB's expertise in RNA biology, a specialization with great potential for innovative translational projects. Prof. Dziembowski has founded and scientifically supervises a business-oriented Genome Engineering Unit, offering the research community a broad range of genetic modifications to mice using CRISPR/Cas9 methodology, along with service to quickly generate transgenic animal models. This entailed development of existing and conceptual thinking of future modern and highly efficient core facilities with services for internal and external customers. With the support of MOSaIC, the IIMCB has also engaged in education of researchers. Together with eight other research institutions specializing in biology, chemistry, medicine and physics, the IIMCB launched a dedicated PhD School: Warsaw-4-PhD. To date, the school has accepted 300 doctoral students from 25 countries. Finally, the Institute has introduced and promotes effective science and HR management, with stress on gender equality and diversity measures.

**The International Institute of Molecular and Cell Biology in Warsaw (IIMCB)** is a research institute in the field of life sciences. It was formally established in 1995 under an international agreement between the government of the Republic of Poland and UNESCO. The IIMCB started its operations in 1999 and has a unique legal status and independence in the Polish scientific system.

**Top quality research**

For years, the Institute has boasted the highest scientific category (A+) in the evaluation of scientific institutions by the Ministry responsible for science. With more than 250 staff members on board, the Institute consists of 14 laboratories, 6 core facilities, and 10 research support units. It follows the best examples of scientific research institutions across the world. Research focuses on studying the molecular mechanisms of human diseases across the levels of biological organization from atoms to organisms (worms, zebrafish, mice).

**The Warsaw PhD School in Natural and BioMedical Sciences** (Warsaw-4-PhD) operates since the 2019/2020 academic year. It is formed by the IIMCB and eight other institutes. The School offers educational and research program for PhD students who are preparing to obtain their degrees in four disciplines: biology, chemistry, physics, and medical sciences. It is the largest institute-based doctoral school in Poland, and it provides fast-growing, interdisciplinary and internationally oriented programme. Admissions to Warsaw-4-PhD occur three times per year and are based on an open international competition. The IIMCB offers their PhD students the opportunity to work in a vibrant, inclusive, and diverse international community, where their research and social needs are fully met.

**Prestigious grants**

Research at the IIMCB is supported by annual subsidies from the Ministry of Education and Science and Polish Academy of Sciences. Still, up to 60% of the yearly institutional budget comes from external competitive sources. Since 2000, our scientists have received 348 grants. Many important ones come from international sources, such as: EU Framework Programmes (ERC AdG and 3 ERC StG grants), Structural Funds through the Foundation for Polish Science, European Molecular Biology Organization, Howard Hughes Medical Institute and EEA and Norway Grants. The IIMCB has also been successful in receiving prestigious grants for institutional development, such as Horizon 2020 ERA Chairs MOSaIC project and Horizon Europe Teaming for Excellence project entitled "*RNA and Cell Biology – from Fundamental Research to Therapies*" (RACE).

**Dynamic growth**

The IIMCB is on its way to unprecedented scientific and institutional growth, in part supported by the RACE project. Through planned expansion from 14 to eventually 20 research groups involved in innovative translational projects in RNA and cell biology, the Institute aims to become a unique Polish center where excellent science profits from state-of-the-art technologies and services provided by its core facilities.

**Career development and working environment**

The environment of the Institute is multinational with English as the working language. The IIMCB implements the policy of:

- international competitions for all research positions, including PhD students,
- open, transparent and merit-based recruitments,
- institutionalized support for foreign candidates, which has resulted in an increase in the number of foreign employees (currently approx. 30%).

**You can be part of our team. Check out our recruitments and join us!**

**New building**

In 2022, the results of the competition for an architectural concept of new building were announced during a ceremonial gala. The winning project envisages 4 above-ground floors and 1 underground floor, with a total building area of over 20,000 m² and a usable space of nearly 14,000 m². As the next step, a building design is prepared, before tendering for a construction company. We are excited about the future of the IIMCB in this attractive and spacious building.

**Visit our website at [www.iimcb.gov.pl](http://www.iimcb.gov.pl)**

# Practical information

## Venue

**IBIB Conference Center**
4 Ks. Trojdena Street
02-109 Warsaw, Poland

## Oral presentation guidelines

The format of the talk should be a maximum of 10 minutes, followed by a 2-minute question-and-answer session.

All speakers are kindly requested to deliver their presentation file to IT Specialist waiting in the Aula before the beginning of the corresponding session. We recommend bringing a presentation file saved on a USB flash drive.

## Poster presentation guidelines

All posters must be displayed in portrait (vertical) orientation. Poster boards accommodate A0 size posters (height 1189 mm, width 841 mm). Please ensure that your display will fit within these parameters. You may choose to go for a smaller size, having regard to the poster legibility. There will be 2 poster sessions:
POSTER SESSION I
numbers 1-35 (September 28, 2023 18:15 - 22:00)
POSTER SESSION II
numbers 36-70 (September 29, 2023 18:15 - 22:00)

Posters should be mounted on the boards before each poster session and removed on the same day.

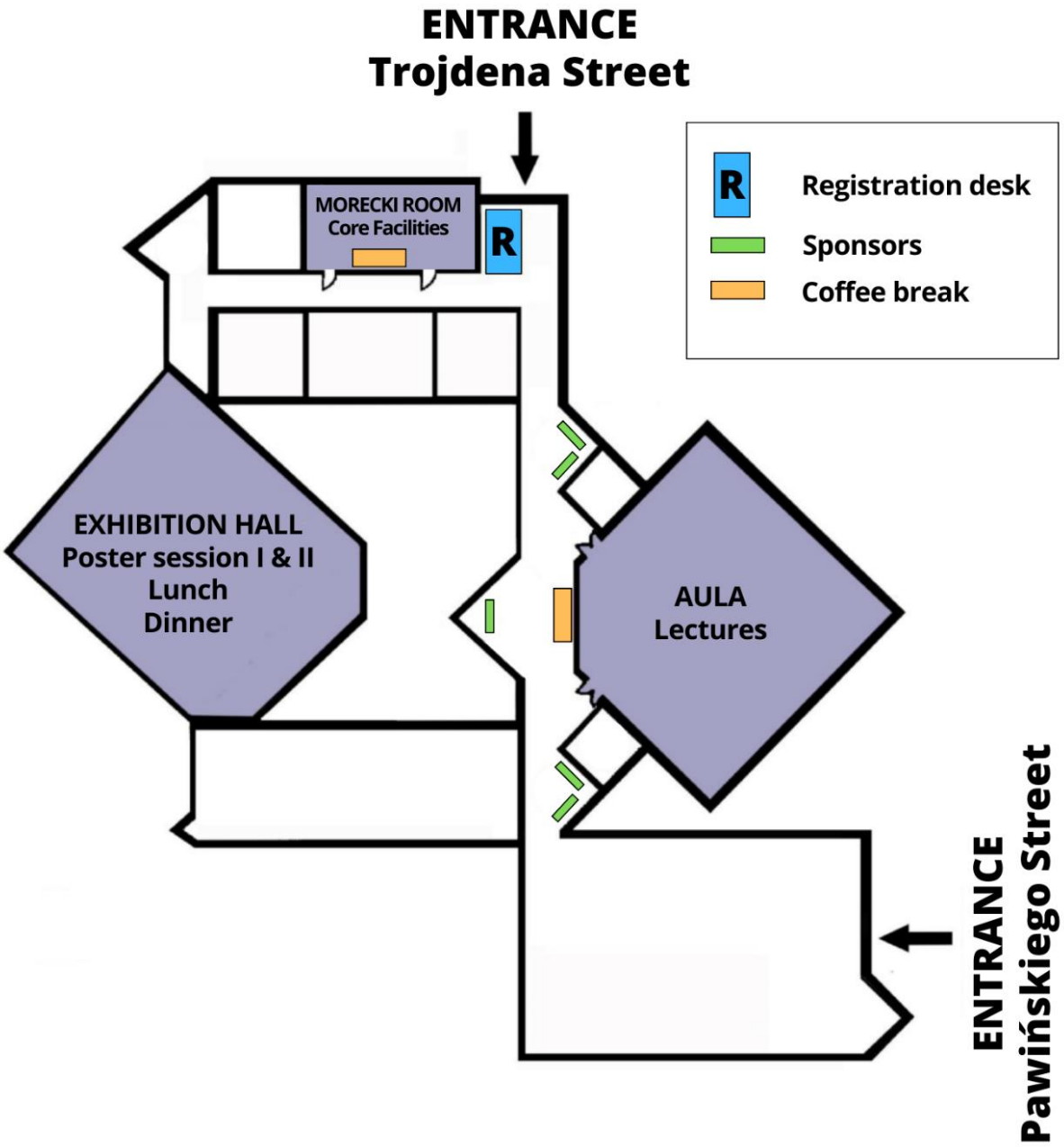
## Registration desk

Registration desk opening hours:
September 28, 2023 08:00-19:00
September 29, 2023 08:45-19:00
September 30, 2023 08:45-12:30

## Conference Internet access

WiFi Access for PLRNA2023 Participants is provided.
Network name: **IBIB-HOTEL**
Password: **zse456yhn**

## Floor plan


ENTRANCE
Trojdena Street
MORECKI ROOM
Core Facilities
R
R
Registration desk
Sponsors
Coffee break
EXHIBITION HALL
Poster session I & II
Lunch
Dinner
AULA
Lectures
ENTRANCE
Pawińskiego Street

# Conference Program

## DAY 01: September 28, 2023 (Thursday)

08:00 - 09:00 Registration

**09:00 - 09:15 Opening**

**09:15 - 10:00 Keynote Lecture**

Chair: **Andrzej Dziembowski**, International Institute of Molecular and Cell Biology in Warsaw, Poland

**Witold Filipowicz**, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
*Traversing the RNA world: lessons from the past and some ideas for the future*

10:00 - 10:30 Coffee break

**10:30 - 12:15 Session I**
Chair: **Dominika Nowis**, Medical University of Warsaw, Poland

**Bertrand Seraphin**, Institute of Genetics, Molecular and Cellular Biology, France
*HELZ2: a new, interferon-regulated, human 3'-5' exoribonuclease of the RNB family is expressed from a non-canonical initiation codon*

**Paweł Sikorski**, University of Warsaw, Poland
*Modifications of mRNA 5'-end defining transcripts as 'self' for innate immune system*

**Magdalena Wołczyk**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*Sequence specificity of RIG-I/IFN signaling*

**Elżbieta Wanowska**, Adam Mickiewicz University, Poznań, Poland
*The role of OIP5-AS1 lncRNA in breast cancer*

**Aleksandra Brouze**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*Immunoglobulin production is enhanced by cytoplasmic polyadenylation mediated by TENT5C acting in concert with FNDC3 proteins*

**Paweł Krawczyk**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*SARS-CoV-2 mRNA vaccine is re-adenylated in vivo, enhancing antigen production and immune response*

**Maria Górna**, University of Warsaw, Poland
*Hold my cap(0): a 5' dependent mRNA capture method to analyze the yeast transcriptome*

**Renata Grzela**, University of Warsaw, Poland
*Crosstalk of cap structure modifications and innate immune response factors*

12:15 - 13:45 Lunch

**13:45 - 14:30** **Keynote Lecture**

Chair: **Kinga Kamieniarz-Gdula**, Adam Mickiewicz University, Poznań, Poland

**Lori Passmore**, MRC LMB, Cambridge, UK
*Molecular machines that regulate mRNA poly(A) tails*

**14:30 - 16:15** **Session II**
Chair: **Gracjan Michlewski**, International Institute of Molecular and Cell Biology in Warsaw, Poland

**Katarzyna Bandyra**, University of Warsaw, Poland
*Polynucleotide phosphorylase - an exoribonuclease and an RNA chaperone in one*

**Lidia Lipinska-Zubrycka**, University of Warsaw, Poland
*Dual role of uridylation in bulk mRNA Decay*

**Daria Riabov**, Research Institute of Molecular Pathology, Austria
*Structure of the recycling human U5 snRNP*

**Tomasz Turowski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
*Co-transcriptional adenylation of nascent RNA polymerase I transcripts*

**Łukasz Szewc**, Adam Mickiewicz University, Poznań, Poland
*The involvement of A. thaliana CFI polyadenylation factor in termination of transcription and U1 snRNP-dependent suppression of premature polyadenylation*

**Szymon Swiezewski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
*Promoter-pervasive transcription pausing Pol II to boost transcription*

**Michaela Ristová**, University of Edinburgh, UK
*A bridge through time: Pin4 links rapid post-transcriptional and transcriptional stress responses to maintain energy homeostasis in S. cerevisiae*

**Sebastian Sacharowski**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
*Uridylation of lncRNAs enhances their chromatin tethering and ability to control seed dormancy through DOG1 gene activation*

16:15 - 16:45 Coffee break

**16:45 - 18:15** **Session III**
Chair: **Magdalena Dziembowska**, University of Warsaw, Poland

**Agnieszka Fiszer**, Institute of Bioorganic Chemistry PAS, Poznań, Poland
*Separation of RNA- and protein-induced pathogenesis in novel Huntington's disease mouse models*

**Anna Baud**, Adam Mickiewicz University, Poznań, Poland
*Identification of RNA binding proteins which influence translational efficiency of toxic polyglycine protein in fragile X-associated tremor/ataxia syndrome*

**Katarzyna Tutak**, Adam Mickiewicz University, Poznań, Poland
*RPS26 a novel RAN translation modifier of RNA harboring expanded CGG repeats in Fragile X-associated syndrome*

**Filip Stefaniak**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*Structural Interaction Fingerprints for analysis of nucleic acid-ligand interactions*

**Tim Kolberg**, Leipzig University, Germany
*Led-Seq - ligation-enhanced double-end sequence-based structure analysis of RNA*

**Vladyslava Liudkovska**, IMoL PAS, Warsaw, Poland
*Dissecting the splicing landscape of human embryonic differentiation*

18:15 - 19:45 Dinner

**18:15 - 22:00 Poster Session I**

## DAY 02: September 29, 2023 (Friday)

08:45 - 09:15 Registration

**09:15 - 10:00 Keynote Lecture**

Chair: **Sebastian Glatt**, Malopolska Centre of Biotechnology, Jagiellonian University in Kraków, Poland

**Alfredo Castello**, MRC-University of Glasgow Centre for Virus Research, Glasgow, UK
*When viral RNA met the cell: a story of protein-RNA interactions*

10:00 - 10:30 Coffee break

**10:30 - 12:15 Session IV**
Chair: **Michał Gdula**, Adam Mickiewicz University, Poznań, Poland

**Marta Sztachera**, Institute of Bioorganic Chemistry PAS, Poznań, Poland
*Investigation of RNA-protein interactions and brain-specific RBPome in the mouse brain tissue*

**Mikołaj Olejniczak**, Adam Mickiewicz University, Poznań, Poland
*RNA recognition by FinO-domain proteins*

**Rafał Mańka**, University of Opole, Poland
*A study of the RNA interactions with membrane vesicles*

**Kishor Gawade**, Adam Mickiewicz University, Poznań, Poland
*FUS modulates the level of ribosomal RNA modifications in health and disease*

**Mateusz Bajczyk**, Adam Mickiewicz University, Poznań, Poland
*The cross-talk between PCF11-similar proteins and CstF64 in flower development in Arabidopsis thaliana*

**Ewa Anna Grzybowska**, Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland
*The spectrum of RNA targets of intrinsically disordered HAX1 protein indicates predominant binding to coding regions and points to the role in ribosome biogenesis and translation*

**Joanna Krupka**, University of Cambridge, UK
*In search of lost ORFs: ultra-sensitive map of noncanonical Open Reading Frames essential for lymphoid cells*

12:15 - 13:45 Lunch

**13:45 - 14:30 Keynote Lecture**

Chair: **Elzbieta Kierzek**, Institute of Bioorganic Chemistry PAS, Poznań, Poland

**Magda Konarska**, IMol PAS, Warsaw, Poland
*Dynamic changes within the RNA catalytic core leading to the second step of splicing*

**14:30 - 16:15 Session V**
Chair: **Marcin Nowotny**, International Institute of Molecular and Cell Biology in Warsaw, Poland

**Maja Cieplak-Rotowska**, IMol PAS, Warsaw, Poland
*Cwc25's role in choosing the right adenosine as the branch site during the first step of splicing*

**Ishani**, IMol PAS, Warsaw, Poland
*Introns defective for the second step of splicing accumulate in the first step spliceosomal conformation, generating dysfunctional, jammed spliceosomes*

**Olga Gewartowska**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*dTag system allows for in vivo studies of essential genes involved RNA metabolism*

**Monika Kwiatkowska**, Institute of Bioorganic Chemistry PAS, Poznań, Poland
*Making zebrafish the dark horse in long noncoding RNA research*

**Klaudia Skrzypek**, Jagiellonian University Medical College, Kraków, Poland
*Potential of SNAIL-dependent small RNAs as regulatory molecules in rhabdomyosarcoma progression*

**Przemysław Płociński**, University of Łódź, Poland
*Bacterial PNPase as a candidate for antimicrobial drug discovery*

**Mario Mörl**, Leipzig University, Germany
*Living in the past: reconstruction of an ancestral tRNA nucleotidyltransferase candidate*

**Agnieszka Kiliszek**, Institute of Bioorganic Chemistry PAS, Poznań, Poland
*Structural studies of small ligands targeting disease-related RNA molecules*

16:15 - 16:45 Coffee break

**16:45 - 18:15 Session VI**
Chair: **Marta Koblowska**, University of Warsaw, Poland

**Leszek Błaszczyk**, Institute of Bioorganic Chemistry PAS, Poznań, Poland
*Exploring long-range RNA interaction in p53 mRNA*

**Kaspar Burger**, University Hospital Würzburg & University of Würzburg, Germany
*NONO nucleolar re-localisation promotes genome stability by shielding nascent transcripts from DNA double-strand breaks*

**Monika Jóźwiak**, Adam Mickiewicz University, Poznań, Poland
*The role of DRH1, RH46 and RH40 in miRNA biogenesis in Arabidopsis thaliana*

**Ewa Stępniak-Konieczna**, Adam Mickiewicz University, Poznań, Poland
*Therapeutic modulation of MBNL1 splicing factor in myotonic dystrophy*

**Agata Stępień**, Adam Mickiewicz University, Poznań, Poland
*Transcription termination in carcinogenesis*

**Monika Zakrzewska-Płaczek**, University of Warsaw, Poland
*Arabidopsis DXO1 affects the processing of precursors of cytoplasmic and chloroplast ribosomal RNA*

**Piotr Gerlach**, IMoL PAS, Warsaw, Poland
*Bunyaviral strategies to reorganize and exploit cellular translation*

18:15 - 19:45 Dinner

**18:15 - 22:00 Poster Session II**

## DAY 03: September 30, 2023 (Saturday)

08:45 - 09:15 Registration

**09:15 - 10:00 Keynote Lecture**

Chair: **Jacek Jemielity**, University of Warsaw, Poland

**Andrea Rentmeister**, Institute of Biochemistry, Department of Chemistry and Pharmacy, University of Münster, Germany
*Optochemical control of mRNA translation*

**10:00 - 10:15 Sponsor Talk**

**Chiara Reggio**, Scale Bioscience / Altium
*Single Cell RNAseq at SCALE: Unlock single-cell gene expression for every researcher and experiment with ScaleBio*

10:15 - 10:45 Coffee break

**10:45 - 12:00 Session VII**
Chair: **Agata Starosta**, Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

**Guillem Ylla**, Jagiellonian University, Kraków, Poland
*The transcriptomics of the evolution of insect metamorphosis*

**Dilek Cansu Gurer**, Izmir Institute of Technology, Turkey
*Genome-wide identification and classification of sisRNAs in human cells*

**Rahul Mehta**, Malopolska Centre of Biotechnology, Jagiellonian University, Poland
*Structural and biophysical characterization of non-coding RNAs*

**Tales Rocha de Moura**, International Institute of Molecular and Cell Biology in Warsaw, Poland
*Structural studies of the Betacoronaviruses 5'-proximal regions*

**Małgorzata Sierant**, Centre of Molecular and Macromolecular Studies PAS, Łódź, Poland
*Intracellular damage of mcm5S2U-tRNA induced by oxidative stress*

**Oleg Dmytrenko**, Helmholtz Institute for RNA-based Infection Research, Germany
*Cas12a2 nucleases form three functionally-distinct clades*

12:00 - 12:15 Awards & closing ceremony

# Keynote Lectures

## Witold Filipowicz

**Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland**

*Traversing the RNA world: lessons from the past and some ideas* for the future

I was fortunate to witness many spectacular discoveries in the area of RNA biology, starting my experimental work in the 1960s when studying medicine in Łódź and working for my PhD in Warsaw. I was also privileged to participate is some early work on eukaryotic translation and mRNA capping during my post-doctoral and sabbatical times with Severo Ochoa and Aaron Shatkin in US in 1970s and early 1980s. This time has been full of many exciting and often unexpected developments in the field of RNA biology, almost as exciting as the discoveries of the last two decades.

My talk will consist of three parts. In the first, I will revisit some early topics of my research; research I have dropped and left inconclusive for different reasons. Were these wise decisions? What happened to this "abandoned" research afterwards? In the second, a lengthier one,

I will discuss a status of the mechanistic studies on miRNA-mediated repression and its regulation, my main interest during last two decades. Finally, I will briefly share some ideas about the future, discussing few examples of current findings in the RNA field, which I find particularly exciting.

## Lori A. Passmore

**MRC Laboratory of Molecular Biology, Cambridge, UK**

*Molecular machines that regulate mRNA poly(A) tails*

The first, and often rate-limiting, step of canonical mRNA decay is removal of the poly(A) tail. There are two conserved deadenylation complexes in eukaryotes: Pan2-Pan3 and Ccr4-Not. In a current model of deadenylation, Pan2-Pan3 removes the distal, and Ccr4-Not the proximal, part of the poly(A) tail. Deadenylation occurs in gene- and context-dependent manners to allow transcript-specific control of poly(A) tail lengths. This targeted deadenylation is important in many processes including the inflammatory response and early development. RNA adapter proteins are thought to target specific mRNAs for deadenylation by Ccr4-Not. Examples of RNA adapters are Pumilio/Puf, TTP/tristetraprolin and the microRNA-induced silencing complex (miRISC). Understanding how specific mRNAs are targeted for deadenylation has been a central question in the field of gene expression for many years. I will present our most recent work on deadenylation.

## Alfredo Castello

**MRC-University of Glasgow Centre for Virus Research, Glasgow, UK**

*When viral RNA met the cell: a story of protein-RNA interactions*

RNA is a central molecule in the lifecycle of RNA viruses, acting not only as messenger (m)RNA but also as a genome. The interactions that viral RNA establishes with the host cell remain, however, largely unknown. My laboratory has developed several approaches to determine systematically and comprehensively the complement of cellular RNA-binding proteins (RBPs) that interact with viral RNA in infected cells. Applied to different viral models, we discovered a new universe of critical host-virus interactions that are largely conserved across viral families. We discovered cellular RNA-binding proteins (RBPs) that accumulate at the viral replication centres upon infection and promote or inhibit viral fitness. In this talk I will present two studies ongoing in my laboratory. The first one focuses on a group of nuclear RBPs that shuttle to the cytosol upon infection to supress viral replication, representing a first line cellular defence against viruses that do not rely on transcriptional activation of the interferon pathway. The second one explores how the cellular RNA degradation machinery plays a crucial role at feeding viral replication by providing free nucleotides. Our results highlight the importance of protein-RNA interactions in the host-virus battlefield, delineating cellular pathways with therapeutic potential for antiviral treatment.

## Magda Konarska

**IMol, Polish Academy of Sciences, Warsaw, Poland**

*Dynamic changes within the RNA catalytic core leading to the second step of splicing*

Splicing removes non-coding sequences (introns) from pre-mRNA to generate mRNA template for protein production. One of many remarkable features of the splicing enzyme, the spliceosome, is its ability to catalyze two different reactions using a single, RNA-based, catalytic center. Whereas the mechanism of the splicing reaction (step) and the arrangement of RNA:RNA interactions at the first step catalytic center has been predicted by genetic experiments and confirmed by cryo-EM structures, the mechanism of the second step still remains elusive. Although the spliceosomal RNA catalytic core visualized by cryo-EM structural analysis appears unchanged throughout the splicing process, genetic experiments in yeast clearly indicate that at least some rearrangements of the catalytic center are needed to reposition substrates for catalysis and adopt the second step catalytic conformation. Using yeast genetic tools, we analyze these spliceosomal rearrangements. I will describe our model for the dynamic changes at the catalytic core, proposing the formation of an unstable, intermediate structure, and subsequent changes leading to second step catalysis. Our model offers new mechanistic insights into splicing reaction, allowing for a better understanding of the selection of a 3' splice site for the second step. Our studies uncover spliceosomal mechanisms controlling splicing fidelity that may lead to new therapeutic strategies for genetic diseases based on analogous mechanisms.

**Andrea Rentmeister**
**Institute of Biochemistry, Department of Chemistry and Pharmacy, University of Münster, Germany**

## *Optochemical control of mRNA translation*

Messenger RNAs (mRNAs) enable the production of almost any functional protein/peptide in the human body as a vaccine or therapeutic agent. The translation of mRNA is a fundamental process in gene expression, and controlling translation is crucial for regulating the production of proteins in cells. However, one cannot control when and where mRNA is translated into protein, which then has a pharmacological effect.

We developed 5' cap analogues with photo-cleavable groups (FlashCaps) that prohibit binding to the eukaryotic translation initiation factor eIF4E and resist cleavage by decapping enzymes.[1] These compounds are compatible with the general and efficient production of mRNAs by *in vitro* transcription. The single photocaging group in FlashCap-mRNAs prevents translation in mammalian cells and in vitro while not raising immunogenicity. The native cap is restored by irradiation, resulting in effective translation. FlashCaps overcome the problem of remaining sequence or structure changes in mRNA after irradiation that limited previous designs. By combining 5' cap analogs with photo-cleavable groups based on coumarin or ortho-nitrobenzyl moieties, multiplexing for two distinct mRNAs was accomplished.[2] Alternative enzymatic routes to photocage mRNAs will be discussed.[3,4] Our results demonstrate that FlashCaps offer a route to regulate the expression of any given mRNA and to dose mRNA therapeutics with spatio-temporal control.

References:

1. N. Klocker, F. P. Weissenboeck, M. van Dulmen, P. Spacek, S. Huwel and A. Rentmeister, *Nat. Chem.* 2022, 14, 905-913.
2. A. Bollu, N. Klöcker, P. Špaček, F. P. Weissenboeck, S. Hüwel and A. Rentmeister, *Angew. Chem. Int. Ed.* 2023, 62, e202209975.
3. L. Anhäuser, N. Klöcker, F. Muttach, F. Mäsing, P. Špaček, A. Studer and A. Rentmeister, *Angew. Chem. Int. Ed.* 2020, 59, 3161-3165.
4. N. Klöcker, L. Anhäuser and A. Rentmeister, *ChemBioChem* 2023, 24, e202200522.

# Lectures

## HELZ2: a new, interferon-regulated, human 3'-5' exoribonuclease of the RNB family is expressed from a non-canonical initiation codon

Eric Huntzinger, Jordan Sinteff, Bastien Morlet, **Bertrand Seraphin**

Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, Illkirch, France

Proteins containing a RNB domain, originally identified in *E. coli* RNase II, are widely present throughout the tree of life. Many RNB proteins are endowed with 3'-5' exoribonucleolytic activity but some have lost catalytic function during evolution. Database searches identified a new RNB domain containing protein in human: HELZ2. Analysis of genomic and expression data with evolutionary information suggested that the human HELZ2 protein is produced from an unforeseen non-canonical initiation codon in Hominidae. This unusual property was confirmed experimentally, extending the human protein by 247 residues that encompass 4 conserved Zn fingers. Human HELZ2 was further shown to be an active ribonuclease despite the substitution of a key residue in its catalytic center. HELZ2 harbors also two RNA helicase domains and several zinc-fingers and its expression is induced by interferon treatment. We demonstrate that HELZ2 is able to degrade structured RNAs through the coordinated ATP-dependent displacement of duplex RNA mediated by its RNA helicase domains and its 3'-5' ribonucleolytic action. The expression characteristics and biochemical properties of HELZ2 support a role for this factor in response to viruses and/or mobile elements.

---

## Modifications of mRNA 5'-end defining transcripts as 'self' for innate immune system

Karolina Drążkowska, Rafal Tomecki, Marcin Warmiński, Natalia Baran, Dominik Cysewski, Anaïs Depaix, Renata Kasprzyk, Joanna Kowalska, Jacek Jemielity, **Paweł J. Sikorski**

Faculty of Biology, University of Warsaw, Poland

A key feature of eukaryotic mRNA is the presence of a 5' cap structure, which is indispensable for several biological processes such as pre-mRNA splicing, mRNA export, and 5' cap-translation. The mRNA cap consists of 7-methylguanosine (m7G) linked by a 5',5'-triphosphate bridge to the first transcribed nucleotide. In mammals, m7G-adjacent nucleotides undergo extensive modifications. Ribose of the first or the first and the second transcribed nucleotide can be subjected to 2'-O-methylation to form cap1 or cap2, respectively. When the first transcribed nucleotide is 2'-O-methylated adenosine, it can be additionally modified to N6,2'-Odimethyladenosine (m6Am). Recently, the crucial role of cap1 in distinguishing between 'self' and 'non-self' transcripts in mammalian cells during viral infection was revealed. Here, we attempted to understand the impact of cap methylations on RNA-related processes. Therefore, we synthesized tetranucleotide cap analogues and used them for RNA capping during in vitro transcription. Using this tool, we found that 2'-O-methylation of the second transcribed nucleotide within the mRNA 5' cap influences protein production levels in a cell-specific manner. This modification can strongly hamper protein biosynthesis or do not influence protein production levels. Interestingly, 2'-O-methylation of the second transcribed nucleotide and the

presence of m6Am as the first transcribed nucleotide serve as determinants that define transcripts as 'self' and contribute to mRNA escape from the host innate immune response. Additionally, cap methylation status does not influence transcript affinity towards translation initiation factor eIF4E or susceptibility to decapping by DCP2 in vitro; however we observe the resistance of cap2-RNA to DXO-mediated decapping and degradation.

## Sequence specificity of RIG-I/IFN signaling

**Magdalena Wołczyk**[1], Jacek Szymański[1], Ivan Trus[1], Agnieszka Bolembach[1], Nila Roy Choudhury[2], Ceren Könüç[1], Zara Naz[1], Elżbieta Nowak[1], Christos Spanos[3], Juri Rappsilber[3,4], Gracjan Michlewski[1,2]

[1] International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Infection Medicine, University of Edinburgh, UK
[3] The Wellcome Centre for Cell Biology, University of Edinburgh, UK
[4] Department of Biotechnology, Technische Universität Berlin, Germany

Viral RNAs and certain Pol III transcripts exhibit a unique feature called 5'-triphosphate (5'-ppp), which activates the receptor RIG-I, triggering an interferon type I (IFN) response. This response plays a vital role in antiviral defense but can also lead to autoimmune diseases if excessively stimulated. While many RNA virus genomes initiate with 5'-ppp adenosine (5'-pppA), higher eukaryotic Pol III transcripts and genomes of certain pathogenic RNA viruses begin with 5'-ppp guanosine (5'-pppG). The reason for this preference is unknown.

New evidence suggests that structured viral and cellular RNAs containing 5'-pppA induce a stronger RIG-I/IFN response compared to RNAs beginning with 5'-pppG. Altering the initial nucleoside from A to G makes viral RNAs nearly undetectable by the RIG-I machinery in human and mouse cells. Similarly, switching the first G to A in Pol III transcripts enhances their immunogenicity. Structural analysis confirms that the RNA pairs have identical conformations, implying that functional disparities cannot be explained by conformational changes. Significantly, RNA pull-down quantitative mass spectrometry reveals several proteins with a specific affinity for 5'-pppA or 5'-pppG transcripts. These proteins likely regulate RIG-I/IFN signalling triggered by viral and endogenous RNAs.

In summary, we demonstrate the sequence specificity of RIG-I/IFN signalling and proposes that 5'-pppG RNAs may help some viruses and Pol III transcripts evade cellular immune sensors. These findings provide insights into the antiviral response against highly pathogenic RNA viruses and the role of Pol III-derived RNAs in autoimmune disorders.

## The role of OIP5-AS1 lncRNA in breast cancer

**Elżbieta Wanowska**, Magdalena Kubiak, Izabela Makałowska, Michał Szcześniak

Institute of Human Biology and Evolution, Adam Mickiewicz University, Poznań, Poland

Long non-coding RNAs (lncRNAs) are RNA molecules longer than 200 nucleotides that are not translated into proteins. They have been found to be critical for a variety of physiological and pathological processes, primarily by regulating gene expression. OIP5-AS1 is an antisense lncRNA to

the known oncogene, OIP5. OIP5-AS1 expression is elevated in several malignancies and is associated with oncogenesis.

Although OIP5-AS1 expression is also dysregulated in breast cancer (BC), its precise role in BC development remains unknown. Recent research has suggested that OIP5-AS1 may facilitate BC proliferation by binding to microRNAs. On the other hand, our recent research shows that OIP5-AS1 affects OIP5 expression in the non-cancerous cell line HEK293 and that the majority of OIP5-AS1 transcripts are maintained in the nucleus. Since it is well known that lncRNAs show different expression patterns in different tissues, we chose to localize OIP5-AS1 in BC cells and discovered that it is associated with chromatin.

This raises two intriguing questions: (i) whether this lncRNA, in addition to acting as a microRNA sponge, can also affect OIP5 expression in cancer cells by acting in the nucleus, and (ii) whether this regulation contributes to the development of BC. Interestingly, we recently knocked down OIP5-AS1 and found that cell viability in BC cell lines was significantly increased compared to non-cancerous cells. Taken together, our results shed light on a novel mechanism of action of OIP5-AS1 and expand the understanding of its function in breast cancer cells.

## Immunoglobulin production is enhanced by cytoplasmic polyadenylation mediated by TENT5C acting in concert with FNDC3 proteins

**Aleksandra Brouze**, Monika Kusio-Kobiałka, Paweł S. Krawczyk, Olga Gewartowska, Andrzej Dziembowski, Seweryn Mroczek

International Institute of Molecular and Cell Biology in Warsaw, Poland

There is growing evidence that 3'end tailing mediated by noncanonical poly(A) and poly(U) polymerases (TENTs) affects the fate of various RNA species in the cytoplasm. Tent5c, a member of the recently discovered family of poly(A) polymerases, is frequently mutated in multiple myeloma, a cancer of terminally differentiated B cells. Notably, TENT5C expression increases after B cell activation. Thus, we aimed to discover the role of TENT5C in B lymphocytes.

Using Nanopore direct RNA sequencing, we performed global profiling of poly(A) tail lengths in wild-type and Tent5c-/- cells, and identified immunoglobulins encoding mRNAs as specific TENT5C substrates. Then, by comparing nuclear and cytoplasmic poly(A) tail lengths, we proved that TENT5C acts in the cytoplasm. Importantly, TENT5C-mediated polyadenylation increases mRNA stability and, as a consequence, immunoglobulin production. Overall, it affects the humoral immune response.

In line with specificity to mRNAs encoding secreted proteins, TENT5C colocalizes with the endoplasmic reticulum. Moreover, it directly affects ER physiology as Tent5c-/- cells have reduced ER volume and expansion dynamics. To elucidate the molecular mechanism of TENT5C substrate recognition, we performed a TurboID proximity labeling experiment. With that, we identified ER-associated FNDC3A and FNDC3B proteins as promising TENT5C interactors. Indeed, with direct RNA sequencing of RNA from Fndc3a- and Fndc3b-silenced cells, we showed that they are necessary for polyadenylation by TENT5C as in their absence, poly(A) tails of immunoglobulins transcripts are not extended.

Concluding, we showed that cytoplasmic polyadenylation by TENT5C is an important regulator of humoral immune response and FNDC3 proteins are necessary for its activity.

## SARS-CoV-2 mRNA vaccine is re-adenylated *in vivo*, enhancing antigen production and immune response

**Paweł S. Krawczyk**, Seweryn Mroczek, Michał Mazur, Olga Gewartowska, Wiktoria Orzeł, Bartosz Tarkowski, Aleksandra Brouze, Wiktor Antczak, Katarzyna Matylla-Kulińska, Sebastian Jeleń, Monika Kusio-Kobiałka, Agnieszka Tudek, Andrzej Dziembowski

International Institute of Molecular and Cell Biology in Warsaw, Poland

Therapeutic mRNA resembles normal mRNA, but is generated through in vitro transcription (IVT), usually in the presence of N1-methyl-pseudouridine (mΨ) to dampen the unwanted innate immune response. The COVID-19 pandemic greatly accelerated the development of such therapeutics, but the knowledge about their metabolism in cells is very fragmentary.

In this study, we comprehensively analysed Moderna's mRNA-1273 COVID-19 vaccine using nanopore direct RNA sequencing (DRS), allowing the identification of full-length sequences with intact poly(A) tails. Interestingly, we revealed that unexpectantly mRNA-1273 ends with mΨCmΨAG nucleotides after the poly(A) tail. We demonstrated that in the model cell line HEK293 mRNA-1273 is swiftly degraded in a process initiated by the removal of mΨCmΨAG, followed by CCR4-NOT mediated deadenylation. In contrast, after intramuscular injection in mice, mRNA-1273 is subjected to more complex modification since it undergoes re-adenylation.

Furthermore, transcriptomic analysis of macrophages, the main group of antigen-presenting cells uptaking vaccine mRNA, revealed that poly(A) tail extension is also observed for multiple endogenous transcripts. This process is mediated by TENT5 poly(A) polymerases. Both macrophages and mice, devoid of TENT5 poly(A) polymerases, produce less antigen encoded by the vaccine. Importantly, lack of TENT5-mediated re-adenylation leads to a severely compromised immune response manifested by a significant drop in specific immunoglobulin production following vaccination in mice.

To sum up, we used DRS to show unexpected properties of mRNA-1273 vaccine and its dynamics in cells. Our findings provide an unexpected principle for the high efficacy of mRNA vaccines and open new possibilities for their improvement.

---

## Hold my cap(0): a 5' dependent mRNA capture method to analyze the yeast transcriptome

Martyna Nowacka, Przemysław Latoch, Matylda Izert, Natalia Karolak, Rafal Tomecki, Michał Koper, Agnieszka Tudek, Agata Starosta, **Maria Górna**

University of Warsaw, Poland

Analysis of the protein-coding transcriptome by RNA sequencing requires either enrichment of the desired fraction of coding transcripts or depletion of the abundant non-coding fraction consisting mainly of rRNA. We propose an alternative mRNA enrichment strategy based on the RNA-binding properties of the human IFIT1, an antiviral protein recognizing cap 0 RNA. Here, we compare for Saccharomyces cerevisiae an IFIT1-based mRNA pull-down with yeast-targeted rRNA depletion by the RiboMinus method. IFIT1-based RNA capture depletes rRNA more effectively, producing high-quality

RNA-seq data with excellent coverage of the protein-coding transcriptome, while depleting cap-less transcripts such as mitochondrial or some non-coding RNAs.

We propose IFIT1 as a cost-effective and versatile tool to prepare mRNA libraries for a variety of organisms with cap 0 mRNA ends, including diverse plants, fungi and eukaryotic microbes.

## Crosstalk of cap structure modifications and innate immune response factors

Jingping Geng, Magdalena Chrabaszczewska, Anna Stankiewicz-Drogon, Karol Kurpiejewski, Marzena Jankowska-Anyszka, Edward Darzynkiewicz, **Renata Grzela**

Faculty of Physics, University of Warsaw, Poland

Different mechanisms are responsible for distinguishing between self and non-self RNAs. Many epitranscriptomic modifications include methylations of bases and riboses near the 5’ end of mRNA. m7GpppNm modification of the cap structure is known as cap 1 while the unmethylated counterpart m7GpppN is cap 0. It has been shown that the stress-inducible protein IFIT1 have an ability to bind the very terminal 5’ end of mRNA depending on its structure modifications. Published reports prove that IFIT1 binding of cap 0 RNA results in inhibition of translation.

We will present kinetic data on the interaction between IFIT1 and short RNAs with different native and synthetic modifications. We will show data on the effects of partner proteins IFIT2 and IFIT3 on the kinetics of IFIT1/RNA interactions. Taken together, these data allowed us to characterize the landscape of 5' end mRNA modifications in terms of recognition by the immune system and translational potential. The data obtained are important for understanding the process of regulation of expression of genetic information, since some modifications of the cap, e.g. 2'-O, modulate the availability of mRNA molecules for translation factors. The data are also relevant to the development of mRNA-based gene therapies. Selecting the right set of 5' end modifications yields transcripts with improved features for efficient production of proteins with therapeutic potential.

## Polynucleotide phosphorylase - an exoribonuclease and an RNA chaperone in one

Tom Dendooven, Ben F. Luisi and **Katarzyna J. Bandyra**

University of Warsaw, Poland

Polynucleotide phosphorylase (PNPase) is an ancient exoribonuclease conserved in the course of evolution and can be found in extant species as diverse as bacteria and humans. In bacteria, where the enzyme is best understood, PNPase acts mainly in RNA degradation and processing. However, bacterial PNPase has been also implicated in RNA stabilisation, because loss of the enzyme leads to degradation of several small non-coding RNAs (sRNAs). It has been shown that E. coli PNPase can associate with some full length sRNAs in vivo and can form a protective complex with sRNAs in conjunction with the RNA chaperone Hfq in vitro. We show the molecular basis of how this enzyme can be re-programmed by Hfq and sRNA to switch from degradative to chaperoning roles in RNA-mediated gene regulation. In the ternary assembly formed by PNPase, Hfq and sRNA, RNA is rerouted away from the enzyme active

site through interactions with Hfq and the KH and S1 RNA binding domains of PNPase. A degenerate ARN-repeat sequence in the RNA substrate interacts with one of the Hfq RNA-binding surfaces, bridging Hfq and PNPase, and indicating a loose sequence preference for carrier assembly. Such complex boosts sRNA stability in vitro by protecting sRNA from cellular ribonucleases like main bacterial endoribonuclease RNase E. These results show how ribonucleoprotein complexes involving RNA chaperones can support RNA-mediated control processes and contribute to their regulatory repertoire.

## Dual role of uridylation in bulk mRNA decay

Maciej Grochowski, **Lidia Lipińska-Zubrycka**, Michal Małecki

Institute of Genetics and Biotechnology, University of Warsaw, Poland

Cytoplasmic messenger RNA (mRNA) turnover is an essential part of eukaryotic gene expression. Bulk mRNA decay is usually initiated by poly(A) tail shortening followed by exonucleolytic degradation catalysed by either 5'-3' exonuclease Xrn1 or one of the 3'-5' exonucleases from the RNase II family. The eukaryotic mRNA is pervasively uridylated at the 3'-end and uridylation is considered to support the 5' to 3' decay. However, uridylation was also shown to protect mRNA from excessive tail shortening or to trigger 3' to 5' decay by U-dependent exonuclease Dis3l2. Those seemingly opposite functions assigned to uridylation prevented defining its exact contribution and its significance to bulk mRNA turnover.

To study the function of uridylation in mRNA metabolism we constructed several fission yeast strains with different decay routes selectively inhibited. We next implemented an effective approach to monitor the RNA 3'-end sequence and estimate non-templated tail length. Unlike other methods, genome-wide 3'-RACE (gw3'-RACE) detects both long as well as very short tails and non-tailed molecules.

Our results support a coherent model of bulk mRNA decay with two functionally different types of uridylation catalysed by different terminal uridyltransferases. The Cid1 TUT-ase is responsible for the uridylation of shortened poly(A) tails while Cid16 uridylates predominantly non-tailed mRNAs. Cid1 uridylation serves two purposes: facilitates Lsm1-7 complex binding and protects mRNA from excessive tail shortening. Both those activities help to inhibit 3' to 5' mRNA decay by the exosome or Dis3L2 exonuclease. Cid16 catalysed uridylation targets deadenylated mRNA for degradation by U-dependent Dis3L2 exonuclease.

## Structure of the recycling human U5 snRNP

**Daria Riabov**, Suppapat Visanpattanasin, Matthias Vorlaender, Laura Fin, Alexander Phillips, Clemens Plaschka

Research Institute of Molecular Pathology, Vienna, Austria

The spliceosome is a multi-megadalton molecular machine that catalyses excision of introns from pre-mRNA. Splicing requires that the spliceosome assembles anew on each intron from five small nuclear ribonucleoprotein particles (snRNPs; U1, U2, U4, U5 and U6) which are recycled after splicing termination to regenerate the components for subsequent splicing rounds. Among the snRNPs, the U5 snRNP forms the 'heart' of the spliceosome, chaperones the formation of the RNA active site, and is substantiality remodeled during splicing.

Despite much progress in the structural understanding of pre-mRNA splicing, how the U5 snRNP is recycled for new splicing rounds remains poorly understood.

To address this gap, we used cryo-electron microscopy (Cryo-EM) to determine the structure of the recycling human U5 snRNP. The structure reveals how the transient U5 snRNP protein CD2BP2 acts at several sites within the U5 snRNP to prepare it for the controlled and ATP-independent joining with the U4/U6 di-snRNP to form the U4/U6.U5 tri-snRNP, the largest building block needed for new spliceosome assembly. Specifically, we show how CD2BP2 may (1) re-position the domains of PRP8, (2) control the binding of PRP6 and TXNL4A, and (3) facilitate its own release through an ATP-independent mechanism.

Taken together, our data show the structure of an isolated and recycling U5 snRNP and suggest mechanisms by which CD2BP2 primes the U5 snRNP for tri-snRNP assembly.

## Co-transcriptional adenylation of nascent RNA polymerase I transcripts

**Tomasz W. Turowski**, Elizabeth Petfalski, David Tollervey

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

RNA polymerase I (RNAPI) efficiently transcribes the large (7 kb) polycistronic pre-rRNA from 150 rDNA loci, forming RNAPI convoys that reduce transcriptional stress. Co-transcriptional binding of key assembly and processing factors promotes structural maturation of the nascent pre-rRNA. Despite surveillance mechanisms to maintain high fidelity, only around 50% of rDNA is transcriptionally active.

Previous studies using UV-crosslinking and high-throughput analysis of cDNA (CRAC) reveal prominent RNAPI density across the first 1000 nucleotides of the transcription unit. The peaks reflect substantial changes in the net transcription rate that inversely correlate with the folding of the nascent transcript. RNAPI elongation is driven by a Brownian ratchet mechanism and prone to frequent backtracking. However, stable folding of the nascent transcript resists backtracking and promotes net elongation.

RNA sequences at the peaks of RNAPI density (sites of minimal elongation rate) preferentially associate with nuclear surveillance complex components (TRAMP and exosome) and carry non-templated oligo(A) tails characteristic of TRAMP activity.

We suggest that sites of slow RNAPI elongation can lead to premature termination and transcript degradation. Oligo(A) addition likely requires RNAPI backtracking, failure of intrinsic RNAPI endonucleolytic cleavage activity, and TRAMP activity. We recreated these steps in vitro and confirmed that oligo(A) addition can occur on backtracked RNAPI.

Application of mathematical modelling has led us to propose a model where transcriptionally inactive rDNA units serve as a buffer for impaired RNAPI transcription, given the high efficiency of rDNA transcription and multiple fail-safe mechanisms.

## The involvement of A. thaliana CFI polyadenylation factor in termination of transcription and U1 snRNP-dependent suppression of premature polyadenylation

**Łukasz Szewc**, Mateusz Bajczyk, Dawid Bielewicz, Susheel Sagar Bhat, Artur Jarmołowski

Institute of Molecular Biology and Biotechnology, Department of Gene Expression, Adam Mickiewicz University, Poznań, Poland

All eukaryotic mature RNAs are produced from RNA Polymerase II primary transcripts that undergo extensive processing, including addition of the cap structure at the 5' end, removal of introns and ligation of exons (splicing), and addition of a poly(A) tail at the 3'end during the cleavage and polyadenylation process. The excision of introns from primary transcripts is catalysed by a highly dynamic and large ribonucleoprotein complex called the spliceosome, composed of U1, U2, U4, U6 and U5 snRNPs, as well as other spliceosomal proteins. Generally, one to one stoichiometry of all spliceosomal snRNPs is needed for spliceosome activity however, in the nucleus U1 snRNP is more abundant than all other snRNPs. This points towards additional functions of U1 snRNP beyond splicing. Indeed, it has been shown in human cells that U1 snRNP is also important for inhibition of premature polyadenylation. This U1 snRNP activity is known as telescripting. Previously, we found and described a similar mechanism in A. thaliana while investigating the role of introns in miRNA genes.

In order to understand the molecular interplay between U1 snRNP and the polyadenylation machinery, we first identified protein partners of the U1 snRNP complex among which the homologue subunits of the human CFIm complex were found. We characterized interactions between the subunits of plant CFI complex (AtCFI25, AtCFI59 and AtCFI68), and discovered that the CFI complex is involved in the alternative polyadenylation in introns, revealing its role in telescripting as previously reported in human cells. Additionally, transcriptome analysis of CFI knockout mutants revealed 3' end extensions of several RNA polymerase II transcripts. These results show that plant CFI polyadenylation factor plays a crucial role in termination of RNA polymerase II transcription.

---

## Promoter-pervasive transcription pausing Pol II to boost transcription

Miguel Montez, Maria Majchrowska, Michał Krzysztoń, Grzegorz Bokota, Sebastian Sacharowski, Magdalena Wrona, Ruslan Yatusevich, Ferran Massana, Dariusz Plewczynski, **Szymon Świeżewski**

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Eukaryotic genomes are pervasively transcribed by RNA polymerase II. Yet, the molecular and biological implications of such a phenomenon are still largely puzzling. Here, we describe noncoding RNA transcription upstream of the Arabidopsis thaliana DOG1 gene, which governs salt stress responses and is a key regulator of seed dormancy. We find that expression of the DOG1 gene is induced by salt stress, thereby causing a delay in seed germination. We uncover extensive transcriptional activity on the promoter of the DOG1 gene, which produces a variety of lncRNAs. These lncRNAs, named PUPPIES, are co-directionally transcribed and extend into the DOG1 coding region. We show that PUPPIES RNAs respond to salt stress and boost DOG1 expression, resulting in delayed germination. This positive role

of pervasive PUPPIES transcription on DOG1 gene expression is associated with augmented pausing of RNA polymerase II, slower transcription and higher transcriptional burst size. These findings highlight the positive role of upstream co-directional transcription in controlling transcriptional dynamics of downstream genes.

## A bridge through time: Pin4 links rapid post-transcriptional and transcriptional stress responses to maintain energy homeostasis in S. cerevisiae

**Michaela Ristová**[1], Vadim Shchepachev[2], Katie Bexley[1], David Tollervey[1]

[1] University of Edinburgh, UK
[2] University of Cambridge, UK

Fluctuating nutrient availability presents an acute challenge to unicellular organisms. We are determining how the yeast Saccharomyces cerevisiae utilises RNA-binding proteins in responding to environmental stress on a second to minute time scale.

Kinetic analyses of the RNA-bound proteome following glucose withdrawal identified ~22 proteins showing rapid, strong changes in RNA association. Amongst the most affected were several almost uncharacterised proteins, including Pin4. Excitingly, we identified Pin4 as key control point for glucose stress that co-ordinates post-transcriptional and transcriptional responses.

We developed a novel UV-crosslinking variant (reCRAC) that allows cell lysis in fully denaturing conditions. reCRAC revealed that Pin4 predominately binds mRNA 3' UTR regions, with enrichment for mRNAs linked to glycolysis and mitochondrial function. This preference is maintained following glucose withdrawal, although overall binding to mRNAs is decreased by 30%. Very unexpectedly, loss of Pin4 almost completely abolished the transcriptional response to glucose starvation, including the induction of stress-specific mRNAs required for energy homeostasis. Deletion of the single RRM domain in Pin4 recapitulated this phenotype, implicating RNA-binding by Pin4 in the transcriptional stress response.

We have shown that translational inhibition after glucose withdrawal is very rapid (<30), whereas elongation is maintained. We postulate that the initial response to glucose withdrawal is metabolic; mediated by very rapid changes in nucleotide concentrations. This is followed by slower changes in signalling pathways, which require RNA binding by Pin4, which acts to bridge post-transcriptional and transcriptional changes needed for energy homeostasis in yeast.

## Uridylation of lncRNAs enhances their chromatin tethering and ability to control seed dormancy through DOG1 gene activation

**Sebastian Sacharowski**, Michał Krzysztoń, Katarzyna Muter, Miguel Montez, Szymon Świeżewski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Uridylation is a posttranscriptional 3'end modification of RNA that consist of non-templated uridines. Uridylation is a widespread posttranscriptional 3'end modification of RNA that affects RNA degradation

in various eukaryotes, including fission yeast, plants, and animals. We propose that the uridylation of plant lncRNAs (long non-coding RNA) is also required for their chromatin tethering.

We identified a chromatin-associated lncRNA, which we named MUSHER, involved in establishing seed dormancy. Unexpectedly, MUSHER is extensively uridylated, and its uridylation impacts RNA's functionality rather than stability. Inactivation of URT1 - main Arabidopsis TUTase - results in alteration of MUSHER polyadenylation without changes in its expression level. Subsequent cellular fractionation analysis showed that the total level of MUSHER did not change in urt1, but the amount of MUSHER attached to chromatin- decreased compared to the nucleoplasmic fraction. Furthermore, we demonstrated that uridylation is important for the chromatin tethering of different lncRNAs.

Uridylation-mediated enhancement of MUSHER chromatin-binding is required for its ability to regulate gene expression. MUSHER activates the expression of DOG1 – a key regulator of dormancy in plants. MUSHER regulates DOG1 alternative polyA site selection by recruiting the CPSF (Cleavage and Polyadenylation Specificity Factor) complex to the proximal polyA region of DOG1.

Our results uncover a new molecular mechanism of lncRNA tethering to chromatin through uridylation, its potential to control gene expression, and its biological importance in regulating seed dormancy.

## Separation of RNA- and protein-induced pathogenesis in novel Huntington's disease mouse models

Magdalena Jazurek-Ciesiołka[1#], Łukasz Przybył[1#], Magdalena Woźna-Wysocka[1#], Paweł Świtoński[1], Joanna Suszyńska-Zajczyk[2], Dorota Wronka[1], Julia Misiorek[1], Grzegorz Figura[1], Adam Ciesiołka[1], Paula Sobieszczańska[1], Maciej Figiel[1], Anna Zeller[3], Magdalena Niemira[3], **Agnieszka Fiszer**[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[2] Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Poland
[3] Genomics and Epigenomics Laboratory, Clinical Research Centre, Medical University of Bialystok, Poland
[#] equal contribution

Huntington's disease (HD) belongs to the neurodegenerative polyglutamine (polyQ) diseases, and is caused by the CAG repeat expansion located in ORF of HTT gene. It is still debated to what extend mutant mRNA-driven disruptions affect HD pathogenesis, as compared to the dominant mechanisms of the mutant polyQ protein gain-of-function.

The aim of this study was to assess the contribution of mutant RNA to the pathogenesis of HD. We generated two HD transgenic mice using knock-in strategy into the Rosa26 locus. These models express one of the two variants of human mutant HTT cDNA fragment, either translated - HD/100Q or non-translated – HD/100CAG, with additional sequences: HA tag and MS2 aptamer, enabling visualization of protein and transcript. The cohorts of animals were analyzed for 21 months with broad spectrum of molecular, behavioral and cognitive methods, at time points every 4 months. Behavioral testing showed a progressive phenotype of created models with more severe phenotype in the HD/100Q model. Rotarod, static rod and open-field tests revealed motor deficits during light phase while ActiMot indicated hyperkinesis during dark phase. Both models also showed some molecular neuropathological

changes in striatum. In conclusion, we demonstrate in vivo evidence for a contributory role of mutant RNA in the pathogenesis of HD.

Acknowledgements of financial support for this study: the National Science Centre, Poland [2012/06/A/NZ1/00094, 2015/19/D/NZ5/02183, 2015/19/B/NZ2/02453 and 2021/41/B/NZ3/03803].

## Identification of RNA binding proteins which influence translational efficiency of toxic polyglycine protein in fragile X-associated tremor/ataxia syndrome

**Anna Baud** and Krzysztof Sobczak

Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland

The premutation expansion of 55-200 CGG (CGGexp) repeats in the 5'UTR of fragile X mental retardation 1 (FMR1) gene, causes fragile X-associated tremor/ataxia syndrome (FXTAS). The CGGexp, if present in mRNA can trigger the repeat associated non-AUG initiated (RAN) translation, which is one of the possible pathogenic mechanisms in FXTAS. Resulting aberrant proteins contain long monoaminoacid tracts (i.e. polyglycine; FMRpolyG), aggregate and accumulate in nuclear inclusions in the brain of FXTAS patients, leading to neuronal death. Despite emerging reports about the possible factors playing role in RAN translation, still little is known about this process.

To identify proteins playing a role in RAN translation in FXTAS, we employed the CGGexp RNA-targeting pull-down approach combined with proteomic analysis, and identified 40 proteins as binding to 5'UTR of FMR1 mRNA in vitro. Selected candidates were further stratified in order to evaluate their contribution to RAN translation efficiency. In cells expressing of FMRpolyG-GFP protein, silencing of insulin-like growth factor 2 mRNA-binding protein (IGF2BP3) led to decrease in production of FMRpolyG protein, but did not decrease the CGGexp-containing mRNA. Additionally, the overexpression of IGF2BP3 resulted in increase of both FMRpolyG protein and mRNA level.

In conclusion, RNA-targeting pull-down approach combined with proteomic analysis allowed us to identify multiple proteins, which bind specifically to the FMR1 5'UTR RNA containing CGGexp . The knock down of selected proteins allowed to evaluate their impact on the production of FMRpolyG. Our results suggests that IGF2BP3 is a novel regulator of RAN translation.

## RPS26 a novel RAN translation modifier of RNA harboring expanded CGG repeats in Fragile X-associated syndrome

**Katarzyna Tutak**, Anna Baud, Krzysztof Sobczak

Adam Mickiewicz University, Poznań, Poland

Short nucleotide repeats are robustly distributed in human genome and contribute to pathogenesis of multiple neurodegenerative disorders such as Fragile X-associated tremor/ataxia syndrome (FXTAS). Pathogenesis of FXTAS is driven by expansion of CGG repeats (CGGexp) in the 5'UTR region of the FMR1 gene. One of the proposed molecular mechanism involved in disease progression is repeat associated

non-AUG (RAN) translation, which results in the production of toxic glycine rich protein (polyG) derived from expanded CGG repeats. Mechanistic insights of RAN translation remain elusive, therefore we aimed to identify novel RAN translation modifiers. We applied RNA-tagging system and conducted mass-spectrometry (MS) based screening which allowed to capture proteins natively bound to FMR1 mRNA containing CGGexp. MS screening revealed a pool of proteins interacting with this RNA in cellulo and among them we selected alternative, small ribosomal subunit called RPS26, and verified its regulatory properties in the context of CGG-related RAN translation in few independent mammalian cells models. Additionally, we investigated the role of chaperone TSR2, which stabilizes RPS26 and enables its incorporation into maturating ribosome. All together, TSR2:RPS26 complex appeared to selectively regulate the level of polyG in mammalian cells and became promising candidate for targeting repeats-associated toxicity in FXTAS.

Funding: Polish National Science Centre: No. 2019/35/D/NZ2/02158 (to Anna Baud) and No. 2020/38/A/NZ3/00498 (to Krzysztof Sobczak).

## Structural Interaction Fingerprints for analysis of nucleic acid-ligand interactions

Natalia A. Szulc[1,2], Zuzanna Mackiewicz[1,3], Janusz M. Bujnicki[1], **Filip Stefaniak**[1]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Laboratory of Protein Metabolism, International Institute of Molecular and Cell Biology in Warsaw, Poland
[3] Laboratory of RNA Biology - ERA Chairs Group, International Institute of Molecular and Cell Biology in Warsaw, Poland

Computational methods play a central role in drug discovery and are widely used in virtual screening, structure optimization, and compound activity profiling. Over the past decades, almost all the attention in medicinal chemistry has been focused on protein-ligand binding, and computational tools have been developed with this goal in mind. With the recent discovery of functional RNAs and their potential applications, RNAs have gained considerable attention as potential drug targets. However, the availability of bioinformatics tools for nucleic acids is limited. Here, we present fingeRNAt-a software tool for detecting non-covalent interactions formed in complexes of nucleic acids with ligands. The program detects nine types of interactions, but the scope of detected interactions can be easily extended using a simple plug-in system. In addition, detected interactions can be visualized using the included PyMOL plugin, facilitating the analysis of molecular complexes at medium throughput. Interactions are also encoded and stored as a bioinformatics-friendly Structural Interaction Fingerprint (SIFt) - a binary string where the corresponding bit in the fingerprint is set to 1 if a particular interaction is present and 0 otherwise. This output format, in turn, enables high-throughput analysis of interaction data using data analysis techniques.

We present applications of fingeRNAt-generated interaction fingerprints for visual and computational analysis of RNA-ligand complexes, including analysis of interactions formed in experimentally determined RNA-small molecule-ligand complexes deposited in the Protein Data Bank. We propose similarity based on interaction fingerprints as an alternative measure to RMSD to recapitulate complexes with similar interactions but different folding. We show the application of SIFts accompanied by machine learning methods to predict the binding of small molecules to RNA and to

facilitate virtual screening experiments. This approach, combined with Explainable Artificial Intelligence (XAI) methods, allows for understanding the decision-making process behind the predictive models.

## Led-Seq - ligation-enhanced double-end sequence-based structure analysis of RNA

**Tim Kolberg**, Sarah von Löhneysen, Iuliia Ozerova, Karolin Wellner, Roland K. Hartmann, Peter F. Stadler, Mario Mörl

Leipzig University, Germany

Structural analysis is an important tool to investigate the function of RNA molecules in vitro as well as in vivo. Several procedures are available, relying on chemical modification inducing RT stops or nucleotide misincorporations during reverse transcription. Other approaches utilize RNA cleavage reactions to generate RT stop signals. However, these methods usually address only one side of the RT stop position. Here, we describe Led-Seq, a new approach based on lead-induced cleavage of RNA, where both resulting cleavage products are investigated. Hydrated lead ions abstract the proton from the ribose 2'-OH group, rendering it highly nucleophilic. As single-stranded RNA regions are more flexible, they exhibit an increased propensity to adopt an in-line conformation, where the nucleophilic 2'-O- attacks and cleaves the neighboring phosphodiester bond. In Led-Seq, the resulting RNA fragments carrying 2',3'-cyclic phosphate or 5'-OH ends are selectively ligated to oligonucleotide adapters. In a subsequent RNA-Seq analysis, cleavage sites are identified as adapter ligation positions. The sequencing reads are transformed into normalized probing signals that are then converted into a probability of a position to be unpaired. With a benchmark set of transcripts in Escherichia coli, we show that Led-Seq is an improved and reliable approach based on metal ion-induced phosphodiester hydrolysis to investigate RNA structures. A great advantage of the double-end analysis is the mutual validation of cleavage sites. Furthermore, it ensures that one of the libraries carries informative reads close to the transcript ends, where reads from the other library are too short for unambiguous mapping.

## Dissecting the splicing landscape of human embryonic differentiation

**Vladyslava Liudkovska**, Phuong Cao Thi Ngoc, Remigiusz Serwa, and Maciej Cieśla

IMol, Polish Academy of Sciences, Warsaw, Poland

Embryonic stem cells (ESCs) are pluripotent cells originating from the inner cell mass of a blastocyst. They possess unlimited self-renewal capacity and the potential to differentiate into any cell type. Maintaining a balance between pluripotency and specification in ESCs is essential for proper embryonic development and evading tumorigenesis. Therefore, the pluripotency-to-differentiation switch in ESCs is tightly regulated through multiple mechanisms. An increasing body of evidence suggests that mRNA splicing and translation play critical roles in determining stem cell fate choices and balancing ESC function.

In our study, we utilized a combination of high-throughput transcriptomic and proteomic approaches to characterize gene expression switches during the exit of human H9 ESCs from the pluripotency. We observed that the abundance of numerous core splicing factors (SFs), particularly those enriched for

U2 and U6 snRNP components, is regulated at the mRNA translation level. Consequently, SF dynamics is associated with altered alternative splicing patterns, indicating that the splicing landscape is re-wired upon ESC differentiation. To identify pluripotency-essential and regulatory SFs and assess their relevance for ESC pluripotency and survival, we employed functional CRISPR-dCas9 interference/activation screening. Subsequent experiments focus on selected SFs to investigate the mechanisms leading to their translational regulation and examine the impact of these mechanisms on the composition of snRNPs and alternative splicing patterns in the context of ESC fate decisions during embryonic development.

## Investigation of RNA-protein interactions and brain-specific RBPome in the mouse brain tissue

**Marta Sztachera**[1], Weronika Wendlandt-Stanek[1], Remigiusz Serwa[2], Monika Piwecka[1]

[1] Department of Non-coding RNAs, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

[2] Proteomics Core Facility (PCF) IMol, Polish Academy of Sciences, Warsaw, Poland

RNA binding proteins (RBPs) are involved in the regulation of RNA's life cycle from transcription through splicing, modifications, transport, stability, translation to degradation and RNA turn-over. Malfunctions of RBPs, their mislocalization and/or distorted RNA-protein interactions have been associated with the pathogenesis of diseases, which is especially recognized in cancer, neurological and neuromuscular disorders. In recent years, the repertoire of proteins that bind RNA expanded greatly. That has been possible due to development of new methods that enable to study RNA-protein complexes in a high-throughput manner. However, the majority of these methods have been set to identify RBPs in cell lines and are focused on protein:mRNA capture. Here, we present our attempts at studying RNA-protein interactome in the mouse brain tissue. We adapted XRNAX method [1] to identify brain-specific RBPome. It makes use of phase separation of RNA, proteins and protein-RNA complexes stabilized by UV-crosslinking, and enables to capture of both non-coding and mRNA-bound proteins. XRNAX protocol followed by mass spectrometry measurements allowed us to capture >400 proteins in the wild-type mouse brain. This XRNAX set of proteins is enriched in known RBPs and revealed >100 proteins that were not previously recognized as RNA binders. We compared our results to the recently published output of eRIC method applied to study mRNA-bound proteins in mouse tissues [2]. Summarizing, XRNAX allowed us to detect RBPs that interact with mRNA and non-poly(A)-tailed RNAs. We identified known and potentially new, unconventional RBPs in the wild-type mouse brain, and these candidate RBPs we are currently validating.

[1] Trendel et al., (2019). The Human RNA-Binding Proteome and Its Dynamics during Translational Arrest. Cell. 176(1-2):391-403

[2] Perez-Perri et al., (2023). The RNA-binding protein landscapes differ between mammalian organs and cultured cells. Nat. Comm. 14, 2027

## RNA recognition by FinO-domain proteins

Maria D. Mamońska, Ewa M. Stein, Maciej M. Basczok, **Mikołaj Olejniczak**

Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland

RNA-binding proteins from the FinO-domain family are present in numerous β- and γ-proteobacteria. Some of the proteins from this family, such as Escherichia coli ProQ, are global RNA binders, while others, including the F-like plasmid FinO protein, only bind few RNAs. Among top RNA ligands of E. coli ProQ are mainly small RNAs and mRNA 3'-UTRs. ProQ binds transcription terminators in its RNA ligands, and competes for its targets against another RNA-binding protein Hfq. ProQ and FinO proteins bind distinct RNAs in E. coli, which raises a question of how the RNA recognition specificity of FinO-domain proteins is achieved.

To explain how E. coli ProQ recognizes RNAs we compared the binding of ten mutants of ProQ containing substitutions in the FinO domain to seven RNAs, which are natural ligands of ProQ. The data showed that while most substitutions uniformly affected RNA binding, two substitutions had a wide range of effects on the binding of these seven RNAs. Further analysis showed that RNA susceptibility to these two substitutions depends on differences in RNA structure at the base of the terminator hairpin. The RNA recognition by FinO-domain proteins was also analyzed by comparing how ProQ and FinO bind to chimeric RNAs consisting of different portions of RNAs specifically recognized by either protein. Together these data show that correct RNA recognition by FinO-domain proteins is determined by RNA structure at the base of transcription terminators, which suggests that RNAs distinctly adjust to the binding pocket in the FinO domain of each protein.

---

## A study of the RNA interactions with membrane vesicles

**Rafał Mańka**, Karolina Sapoń, Teresa Janas, Tadeusz Janas

Institute of Biology, University of Opole, Poland

Exosomes are small membrane vesicles that originate from endosomal membranes. Being released from cells into the extracellular environment, exosomes may transport various cargo such as RNA, DNA, proteins or lipids.

RNA loading into exosomes is a complex process that still is not fully understood. Researchers identified certain nucleotide motifs (EXO-motifs) within isolated exosomal RNAs that may be involved in the RNA loading process. Other studies point to the presence of specific domains (lipid rafts) within lipid membranes that may regulate RNA-membrane interactions.

In this study, the impact of the following factors was analysed in the RNA-membrane interactions: sequence motifs of RNA and a presence of lipid rafts in the vesicles membranes as well as some RNA's secondary structure motifs.

A group of RNAs containing various RNA motifs as well as liposomes both with and without raft domains were used for the RNA- membrane affinity analysis.

To assess the degree of RNA-membrane interactions, fluorescence measurements were applied based on the FRET mechanism. The dissociation constant was calculated, showing the degree of the RNA affinity to the lipid membrane.

Results showed a significant difference in the RNA-membrane affinity depending on the membrane composition (the presence of lipid rafts favoured RNA-to-membrane affinity). Also some RNA sequence motifs, such as the CCCU sequence, seem to support RNA-membrane interactions, as well as some structural motifs of RNA secondary structure, such as the presence of a small loop. In contrast, a long single-stranded ending within RNA structure appears to diminish RNA affinity to the raft vesicles.

## FUS modulates the level of ribosomal RNA modifications in health and disease

**Kishor Gawade**, Patrycja Plewka, Sophia Julia Häfner, Anders Henrik Lund, Virginie Marchand, Yuri Motorine, Michal Wojciech Szczesniak, Katarzyna Dorota Raczynska

Adam Mickiewicz University, Poznań, Poland

FUS is a multifunctional protein involved in many aspects of RNA metabolism. In this study, we show that FUS depletion results in a change of expression of numerous host genes and corresponding intronic small nucleolar RNAs (snoRNAs) that guide 2'-O methylation and pseudouridylation of specific positions in ribosomal RNAs (rRNAs) and small nuclear RNAs (snRNAs). Using RiboMeth-seq and HydraPsiSeq for profiling site-specific 2'-O-methylation and pseudouridylation of rRNAs, we demonstrated considerably higher modification at several sites in HEK293T and SH-SY5Y cells with FUS knockout (FUS KO) compared to wild-type cells. We observed a similar direction of changes in rRNA modification in differentiated SH-SY5Y cells with the FUS mutation (R495X) related to the early-onset disease phenotype of amyotrophic lateral sclerosis (ALS). Furthermore, the modification pattern of some rRNA positions was correlated with the abundance of corresponding guide snoRNAs in FUS KO and FUS R495X cells. Our findings reveal a new role for FUS in modulating the modification pattern of rRNAs, possibly contributing to generating ribosome heterogeneity that may constitute a finetuning mechanism for translation efficiency/fidelity. Therefore, we suggest that a site-specific increase in the levels of 2'-O-Me and pseudouridylation in rRNAs from cells with the ALS-linked FUS mutation may represent a new translation-related mechanism that underlies disease development/progression.

## The cross-talk between PCF11-similar proteins and CstF64 in flower development in Arabidopsis thaliana

**Mateusz Bajczyk**, Łukasz Szewc, Dawid Bielewicz, Monika Jóźwiak, Zofia Szweykowska-Kulińska, Artur Jarmołowski

Adam Mickiewicz University, Poznań, Poland

Eukaryotic messenger RNA (mRNA) is produced from the primary transcript (pre-mRNA) through extensive processing steps, including splicing, 5' cap and 3' poly-A tail addition. The poly(A) tail protects mRNA from degradation, and it is required for translation initiation. About 70% of Arabidopsis genes have more than one polyadenylation site and alternative polyadenylation (APA) can change the length and the composition/information encoded in the mature transcript. In plants, one of the best-studied

processes affected by APA is the control of flowering time. In Arabidopsis thaliana PCF11-similar protein 4 (PCFS4), homolog of yeast polyadenylation factor protein 11 (PCF11), is an important factor involved in this process. Knock-out pcfs4 mutants exhibit a delay in flowering time. However, in A. thaliana there are three additional PCF11-similar proteins: PCFS1, PCFS2, and PCFS5, which are not characterized. We showed that pcfs2 mutant leads to an opposite effect then pcfs4 mutant - accelerating flowering time. Interestingly, some of the double mutants showed more severe phenotypes including aberrant development of male part of flowers. This phenotype resembles mutant of different polyadenylation factor CstF64. Using FRET-FLIM method we showed that all A. thaliana PCF11-similar proteins interact with CstF64. Using PAT-seq (Poly(A)-Tag Deep Sequencing) we showed that in pcfs as well as cstf64 mutants in comparison to wild-type plants distal polyadenylation site is mostly chosen. Moreover, we noticed that in analyzed mutants all genes encoding pollen coat proteins have altered polyadenylation which strongly suggests that polyadenylation changes affect male flower development.

This work was supported by National Science Center 2015/19/N/NZ1/01997.

## The spectrum of RNA targets of intrinsically disordered HAX1 protein indicates predominant binding to coding regions and points to the role in ribosome biogenesis and translation

Anna Balcerak, Ewelina Macech-Klicka, Maciej Wakula, Rafal Tomecki, Krzysztof Goryca, Malgorzata Rydzanicz, Mateusz Chmielarczyk, Malgorzata Szostakowska-Rodzos, Marta Wisniewska, Aleksandra Helwak, Grzegorz Kudla, **Ewa A. Grzybowska**

Maria Sklodowska-Curie National Research Institute of Oncology, Warsaw, Poland

HAX1 is a multifunctional protein involved in the regulation of crucial processes: apoptosis, regulation of calcium homeostasis, cell migration, mitochondrial proteostasis. However, the exact mechanisms of HAX1 actions in the cell remain elusive and its functionality probably depends on its disordered structure. HAX1 RNA-binding abilities are known for some time, but up to now, only two transcripts were identified. Using high-throughput techniques (RIP-seq and CRAC) our group characterized the whole spectrum of the potential HAX1 RNA targets and confirmed several of them. The main novelty of the obtained results consist in the observation that the binding occurs mainly in coding regions of the analyzed transcripts, and not, as was expected, in their 3'UTR regions. It was shown (on selected examples) that CDS binding protects the transcripts from degradation. The second most important conclusion is that the high proportion of the analyzed targets belong to the transcripts encoding proteins involved in ribosome biogenesis and translation. Interestingly, many of the non-coding (intronic) regions identified in the screen encoded sno-RNA, additionally pointing to the role in ribosome biogenesis. Subsequently, it was shown, that HAX1 deficiency affected ribosome profile, namely 40S:80S ratio. The results reveal the unknown functionality of HAX1 protein, including protective CDS binding and (subsequent?) regulation of ribosome biogenesis and translation, which may contribute to the explanation of HAX1 role in diseases (neutropenia and cancer).

## In search of lost ORFs: ultra-sensitive map of noncanonical Open Reading Frames essential for lymphoid cells

**Joanna A. Krupka**, Chun Gong, Jie Gao, Stamatia Vori, Hendrik Runge, Shamith Samarajiwa, Daniel J. Hodson

University of Cambridge, UK

Recently, the topic of hidden proteome has attracted much interest in the context of immunity and oncogenesis. To map the position of translating ribosomes, we prepared and analysed a collection of almost 80 Ribo-Seq libraries from lymphoid cell lines and human lymphocytes. We boosted the sensitivity of open reading frames (ORFs) identification by using 4 algorithms and adopting a hierarchical merging strategy, which resulted in using up to 668 mln Ribo-Seq reads in a single run. We identified 43537 novel ORFs, of which 13.6% were on noncoding transcripts. 10% of all unique proteins quantified in mass spectrometry were assigned to the novel ORFs. Then, we deployed a custom CRISPR-Cas9 library targeting the 1,600 highest confidence ORFs in 6 cell lymphoid lines. Strong dropout was seen across all cell types analysed for more than 100 novel ORFs, suggesting that their translated micropeptides may be essential for lymphocyte survival. The effect size was comparable to the knock-out of well-known genes essential for B-cells, such as MYC. Induction of apoptosis and growth disadvantage were confirmed when selected gRNAs were validated individually. ORFs protein expression, cellular localisation and mechanism of action is currently being investigated. Although the function these essential ORFs is yet to be determined, many show strong evolutionary conservation and high level of disorder, suggesting that they may act as interaction hubs or participate in cell compartmentalisation.

---

## Cwc25's role in choosing the right adenosine as the branch site during the first step of splicing

**Maja Cieplak-Rotowska** and Magda Konarska

IMol, Polish Academy of Sciences, Warsaw, Poland

Within the conserved yeast branch site (BS) consensus UACUAAC, only the second of the two As is selected for catalysis, however, the basis for this preference is unknown. The BS motif UACUAAC forms a duplex with the conserved U2 snRNA motif GUAGUA, from which the BS-A must be bulged out to catalyze the first step of splicing. Mutations of the BS can affect the 3'SS selection, with severe consequences for splicing specificity. Thus, it is critical to know how the spliceosome selects this adenosine as the branch site.

Cwc25 is an essential spliceosomal protein required for the first step of splicing. In cryo-EM structures, the N-terminus of Cwc25 protrudes into the BS-U2 duplex. This N-terminus is positively charged except for position D5. We propose that the D5 negative charge pushes away the phosphodiester backbone of the BS-A, contributing to its bulging and selection as the branch site.

We interrogated the D5 position for the use of neighboring nucleotides as branch sites. We use the BS-AAA reporter with a modified UACUAAa branch site motif, providing an additional A as a possible branch site. Our analysis of the N-terminus of Cwc25 has demonstrated that both the position and charge of D5 contribute to splicing. Analogous analysis of the Cwc25-S3 position did not result in any

detectable splicing effects. The ongoing primer extension experiments will confirm positions of selected BS-A.

These results support the model of Cwc25's role in the BS-A selection by the D5 negative charge contributing to BS-A positioning for catalysis.

## Introns defective for the second step of splicing accumulate in the first step spliceosomal conformation, generating dysfunctional, jammed spliceosomes

**Ishani** and Magda Konarska

IMol, Polish Academy of Sciences, Warsaw, Poland

A series of conformational rearrangements of the spliceosome affects its assembly, catalysis, and disassembly steps. Although splicing specificity is largely determined during the spliceosome assembly, modulation of spliceosome conformations may also alter splicing catalysis of suboptimal introns, affecting splicing fidelity. We observed that overexpression of ACT1/CUP1 reporters with second (but not first) step defects (e.g. 3'SS mutants) results in significant growth inhibition of wild-type yeast strains. Such introns are defective in the transition into the 2nd step conformation. Indeed, overexpression of Prp16 ATPase, which promotes transition from the 1st-to-2nd step catalytic conformation, corrects growth defects of such mutant introns. These results indicate that 3'SS mutants inhibit transition to the 2nd step catalytic conformation, accumulating spliceosomes in the 1st step conformation.

In addition, we showed that the expression of 3'SS mutants from a mutant form of the strong GPD promoter relieves cell growth inhibition. Similarly, 3'SS intron mutant expression from low copy number plasmids improves growth rate as compared to expression from high copy number plasmids. Finally, the expression of additional copies of U2 and U6 snRNAs also correct growth defects. These results suggest that overexpression of reporter pre-mRNA generates dysfunctional, jammed spliceosomes, saturating the endogenous pool of spliceosomal components. This results in splicing inhibition of endogenous introns, ultimately inhibiting cell growth. Overall, our findings shed light on possible mechanisms of modulating splicing fidelity in yeast.

## dTag system allows for in vivo studies of essential genes involved RNA metabolism

Wiktor Antczak, Marcin Szpila, Katarzyna Sałas, Andrzej Dziembowski, **Olga Gewartowska**

International Institute of Molecular and Cell Biology in Warsaw, Poland

CRISPR/Cas9 methodology has revolutionized the generation of genetically modified organisms. From an engineering point of view, creating knock-out animals is no longer a challenge. However, constitutive gene knockouts of essential genes, such as genes involved in RNA metabolism, often result in lethality. It is estimated, that generation of homozygotic knock-out animal is impossible for approximately 30% of the genes.

To overcome this issue, we have implemented dTag system that allows for the depletion of proteins in a time-controlled manner. In this system, a small degrader tag is added to the endogenous protein but remains inactive until the addition of the ligand. Once the ligand is added, the fusion protein is ubiquitinated and degraded. Using CRISPR/Cas9 method we have generated a dozen of different dTagged mouse lines. All targeted genes were embryonic lethal in as homozygotic KOs or displayed severe harmful phenotype. Efficiency of generation is very high, around 13% of pups born after microinjections had a correct mutation. In most cases the addition of the tag had no effect on embryo survival or mice well-being. After addition of the ligand depletion of the tagged protein was fast and efficient both in vitro and in vivo.

We show a very efficient method for the generation of knock-in animals with a degrader tag. Such models can open new opportunities to study essential genes with knockout are embryo-lethal or display harmful phenotype. Implementing such models could open opportunities for novel research and improve laboratory animals' welfare.

## Making zebrafish the dark horse in long noncoding RNA research

**Monika Kwiatkowska**[1,2], Tugce Kocamanoglu[1], Daniel Kuźnicki[1], Sílvia Carbonell-Sala[3], Roderic Guigo[3,4], Barbara Uszczyńska-Ratajczak[1]

[1] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[2] International Institute of Molecular and Cell Biology in Warsaw, Poland
[3] Centre for Genomic Regulation, Barcelona, Catalonia, Spain
[4] Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain

Vertebrate genomes produce thousands of long noncoding RNAs (lncRNAs) - RNA molecules longer than 200 nucleotides with limited protein coding potential. Although a growing number of lncRNAs is linked to crucial biological processes, >97% of them remain functionally uncharacterized. Application of animal models helps to understand biological roles of lncRNAs. However, this exploration heavily depends on the quality of genome annotations. While zebrafish (Danio rerio) has emerged as a powerful and promising vertebrate model for exploration of lncRNA biology, its genome annotation lag far behind those for human or mouse, significantly hampering its usage. To advance towards a complete and accurate catalogue of lncRNA genes in the zebrafish genome, we established the CapTrap-nanoCLS protocol. CapTrap is a library preparation method which by incorporation of 5'-Cap selection step specifically enriches full-length transcripts with simultaneous and effective depletion of rRNA molecules (<0.25%). Additionally, to boost the detection of lowly represented lncRNAs, CapTrap was combined with nanoCLS approach – a targeted RNA sequencing method that connects RNA capture and long-read Nanopore sequencing. RNA capture probes efficiently fished for lncRNAs, offering 48.4-fold enrichment of targeted sequences and ~15 times higher detection of novel genes in the intergenic space in post-capture libraries. Implementation of CapTrap-nanoCLS resulted in discovery of novel transcript isoforms and lncRNA loci in zebrafish. Our improved zebrafish genome annotation will definitely increase the biological significance of zebrafish as an animal model for modeling gene function and will facilitate evolutionary conservation studies of lncRNAs beyond mammals.

## Potential of SNAIL-dependent small RNAs as regulatory molecules in rhabdomyosarcoma progression

**Klaudia Skrzypek**, Karolina Bąk, Marta Kot, Paweł Konieczny, Marcin Majka

Jagiellonian University Medical College, Kraków, Poland

Rhabdomyosarcoma (RMS) is a frequent mesenchymal tumor of soft tissue that causes death and morbidity of children and adolescents. RMS originates from an impaired differentiation of myogenic progenitors. One of crucial regulators of RMS progression is the SNAIL transcription factor. In our previous studies, we demonstrated that SNAIL silencing completely abolished the growth of human RMS xenotransplants in mice. Furthermore, we discovered that SNAIL-microRNAs axis is a crucial regulator of rhabdomyosarcoma growth. The aim of our current studies was to further investigate the potential of SNAIL-regulated small RNAs as regulators of rhabdomyosarcoma progression.

To investigate the effect of SNAIL silencing in RMS on small RNA transcriptome, the next generation sequencing was performed. We discovered statistically significant differences in expression level of several small nucleolar RNAs. Further investigation of their action in future may reveal their regulatory roles in RMS.

Importantly, we have also discovered that RMS cells can spontaneously uptake high levels of small RNAs, such as siRNA labeled with fluorescent dye, without necessity of any transfection reagent. Furthermore, RMS cells can secrete small RNAs, such as SNAIL-regulated microRNAs in exosomes. The selected microRNAs secreted by tumor cells may enter endothelial cells and regulate their function, which suggests their potential to regulate tumor vascularization.

To conclude, we discovered small nucleolar RNA candidates for further research in RMS and we suggested the role of small RNAs uptake by tumor and endothelial cells in regulation of RMS progression.

This work was supported by UJCM – N41/DBS/000851 and NCN in Poland: 2018/29/B/NZ5/00915.

---

## Bacterial PNPase as a candidate for antimicrobial drug discovery

Jaroslav Lavrynchuk[1], Jakub Skibiński[1], Marcin Włodarczyk[1], Urszula Zarzecka[1], Agnieszka Stepasiuk[2], Irena Bylińska[2], Jarosław Dziadek[1], Magdalena Chmiela[1], **Przemysław Płociński**[1]

[1] University of Łódź, Poland
[2] University of Gdańsk, Poland

RNA decay is an essential process that could be targeted by novel classes of antimicrobial drugs or by repurposing existing drugs. Knowing that RNA degradosome constituents contribute to antibiotic resistance in key human pathogens, like Mycobacterium tuberculosis (Mtb), we set out a study to find specific inhibitors of bacterial polynucleotide phosphorylase enzymes - PNPases.

We have purified large quantities of Mtb and Helicobacter pylori (Hpy) PNPase to perform parallel drug screening of enzymes that are either essential (PNP Mtb) or dispensable (PNP Hpy) for the growth and survival of human bacterial pathogens. We have optimized a medium/high-throughput assay to access the phosphorolytic or polymerization activities of PNPase on oligo RNA substrates in the presence of fluorescent Thioflavin T. Having these tools on hand, we have then performed a screening of potential PNPase inhibitors with Bioactive Library Plus (MedChemExpress, NJ, USA), finding some promising hits that we aim to further develop in collaboration with synthetic chemists.

We believe that, despite PNPase conservation between humans and bacteria, we will be able to discover a substance or a group of substances that will allow us to inhibit bacterial enzymes with high specificity. Finding such bioactive substances could lead to the development of potent future antibiotics that will aid the fight against deadly superbugs.

## Living in the Past: Reconstruction of an ancestral tRNA Nucleotidyltransferase Candidate

Martina Hager, Marie-Theres Pöhler, Franziska Reinhardt, Karolin Wellner, Jessica Hübner, Heike Betat, Sonja Prohaska, **Mario Mörl**

Institute for Biochemistry, Leipzig University, Germany

The addition of the CCA terminus at the tRNA 3'-end is an efficient and highly accurate polymerization reaction catalyzed by tRNA nucleotidyltransferase. To get insights into the evolution of this highly conserved and ubiquitous RNA synthesis, the reconstruction and characterization of ancestral enzyme candidates is a versatile tool. Here, we investigate a reconstructed candidate of a CCA-adding enzyme from Gammaproteobacteria, representing an activity that presumably existed about 2 billion years ago. The recombinant enzyme is catalytically active over a wide temperature range and exhibits a remarkably high polymerization fidelity in vitro as well as in vivo, indicating that error-free CCA-synthesis already existed at this early stage of evolution. Surprisingly, and in contrast to modern CCA-adding enzymes, the ancestral candidate binds its tRNA substrate with a higher affinity. Our data indicate that this enhanced substrate binding interferes with product release, resulting in a low turnover number of the reconstructed enzyme. Accordingly, it seems that the weaker tRNA binding of modern CCA-adding enzymes is the mechanistic cause for their higher catalytic activity. Due to these differences in substrate binding, the ancestral candidate seems to catalyze a processive CCA-addition, while modern enzymes work in a more distributive way.

## Structural studies of small ligands targeting disease-related RNA molecules

Leszek Błaszczyk, Martyna Mateja-Pluta, Marcin Ryczek, Ronald Micura, Kazuhiko Nakatani, **Agnieszka Kiliszek**

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

The presented work is a part of our crystallographic studies focused on structural analysis of interactions between disease related RNA and synthetic molecules. Although the recent progress in high-throughput screening of small molecule libraries resulted in discovery of a number of drug-like

compounds their further improvement requires determination of three-dimensional structures unravelling the details of RNA-ligand interactions.

We will present crystallographic studies of complexes of small ligands and RNAs associated with neurodegenerative disorders called TREDs (Trinucleotide Repeat Expansion Disorders). The abnormal expansion of repeated sequences located within certain genes results in mutated mRNAs gaining pathogenic properties. The specific bindng of small molecules to mutated RNA can block pathological pathways preventing disease progression. We analysed a series of small molecules recognizing unique pattern of nucleobases engaged in non-canonical pairing or located in single stranded regions of repeated RNA sequences. Structures of the complexes allowed detailed characterization of interactions between the ligand and RNA indicating how small compounds can be improved for future biomedical studies.

This research was funded by National Science Centre, Poland, UMO-2022/45/B/NZ7/03543 and UMO-2017/26/E/NZ1/00950.

## Exploring long-range RNA interaction in p53 mRNA

Agnieszka Kiliszek, Wojciech Rypniewski, **Leszek Błaszczyk**

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

p53 protein is a key regulator of cellular homeostasis by coordinating framework of anti-proliferative pathways as a response to various stress factors. Although the main mechanism of stress-dependent induction of p53 protein relies on posttranslational modifications influencing its stability and activity, a growing number of evidences suggest that complex regulation of p53 expression occurs also at the mRNA level. This study explore structural determinants of long-range RNA-RNA interaction in p53 mRNA, crucial for stress-dependent regulation of p53 protein translation. We demonstrate that the eight nucleotide bulge motif plays a key structural role in base pairing of complementary sequences from the 5' and 3' untranslated regions of p53 mRNA. We also show that one of the p53 translation regulators, nucleolin, displays an RNA chaperone activity and facilitates the association of sequences involved in the formation of long-range interaction in p53 mRNA. Nucleolin promotes base pairing of complementary sequences through the bulge motif since mutations of this region reduce or inhibit pairing while compensatory mutations restore this interaction. Mutational analysis of nucleolin reveal that all four RNA recognition motifs are indispensable for optimal RNA chaperone activity of nucleolin. These observations help to decipher the unique mechanism of p53 protein translation regulation pointing bulge motif and nucleolin as the critical factors during intramolecular RNA-RNA recognition in p53 mRNA.

This study was supported by National Science Centre (Poland) [UMO-2016/23/D/NZ1/02565].

## NONO nucleolar re-localisation promotes genome stability by shielding nascent transcripts from DNA double-strand breaks

Barbara Trifault[1,2], Victoria Mamontova[1,2], Giacomo Cossa[2], Julia Hofstetter-Hadry[2], Sabina Ganskih[3], Yuanjie Wei[3], Pranjali Bhandare[2], Apoorva Baluapuri[2], Daniel Solvie[2], Carsten P. Ade[2], Peter Gallant[2], Mathias Munschauer[3], Elmar Wolf[1,2], Martin Eilers[1,2], **Kaspar Burger**[1,2]

[1] Mildred Scheel Early Career Center for Cancer Research, University Hospital Würzburg, Germany
[2] Department of Biochemistry and Molecular Biology, Biocenter of the University of Würzburg, Germany
[3] Helmholtz Institute for RNA-based Infection Research, Helmholtz Center for Infection Research, Würzburg, Germany

RNA-binding proteins (RBPs) are critical for gene expression and emerge as effectors of the DNA damage response (DDR). The non-POU domain-containing octamer-binding protein NONO/p54nrb is a multifunctional RBP and often deregulated in cancer. NONO marks nuclear paraspeckles for editing and retention of messenger (m)RNA in unperturbed cells, but also undergoes re-localisation to the nucleolus upon induction of DNA double-strand breaks (DSBs). However, the relevance of NONO nucleolar re-localisation for genome maintenance is poorly understood.

Here we show that the topoisomerase-II inhibitor etoposide stimulates the production of RNA polymerase II (RNAPII)-dependent, DNA damage-induced nucleolar antisense RNAs (diNARs) in human cancer cells. diNARs originate from distinct nucleolar intergenic spacer (IGS) regions and form DNA-RNA hybrids (R-loops) to tether NONO to the nucleolus. NONO nucleolar re-localisation depends on its RRM1 domain and is accompanied by a NONO-dependent increase in nascent transcripts in the nucleolus. NONO occupancy at protein-coding gene promoters, in turn, is reduced by etoposide, which attenuates pre-mRNA synthesis and modulates R-loop levels. The depletion of NONO prior to DNA damage triggers elevated levels of DSB-specific histone H2B lys-120 acetylation marks and prolongs DSB signaling. Together, we describe a novel DDR pathway that engages the nucleolus to detain NONO and aberrant transcripts from broken chromatin, which facilitates efficient DSB repair. Our data suggest that the interference with the nucleolar DDR may offer a novel therapeutic strategy in tumours that express elevated levels of NONO.

---

## The role of DRH1, RH46 and RH40 in miRNA biogenesis in Arabidopsis thaliana

**Monika Jóźwiak**, Mateusz Bajczyk, Dawid Bielewicz, Zofia Szweykowska-Kulińska, Artur Jarmołowski

Adam Mickiewicz University, Poznań, Poland

MicroRNAs (miRNAs) are short, single-stranded, and non-coding RNAs that regulate gene expression at the post-transcriptional level. The process of miRNA production is complex and involves many proteins. Recently, one of the extensively studied group of proteins involved in miRNA biogenesis are DEAD-box helicases. The Arabidopsis thaliana genome contains 56 DEAD-box helicases. Among them,

only DRH1, RH46 and RH40 contain WW domains that have been identified as a site for interaction with the CTD domain of RNA Polymerase II (RNAPII) using in silico analysis. Moreover, it was shown that DRH1 is engaged in rRNA processing, nonsense-mediated decay (NMD) and mRNA export. However, the role of DRH1, RH46 and RH40 in miRNA biogenesis is unknown.

During our research, we tested single, double and triple Arabidopsis mutants of DRH1, RH46 and RH40 in standard (22°C) and changed growth conditions (16°C). We observed that the lack of all studied helicases affects miRNA levels when cultivated at both temperature conditions. Moreover, the drh1 rh46 rh40 phenotype was changed compared to wild-type plants when cultivated at 16°C. Using FRET-FLIM analysis we showed that DRH1 directly interacts with SERRATE (SE) and ARGONAUTE1 (AGO1), two important proteins in the miRNA biogenesis pathways. Furthermore, DRH1, in line with in silico predictions, interacts with the CTD domain of RNAPII. However, we observed the interaction between DRH1 and the CTD domain even when the WW domain was removed. Thus, our result suggests that the WW domain is not important for the DRH1-CTD interaction.

## Therapeutic modulation of MBNL1 splicing factor in myotonic dystrophy

Nikola Musiała, Gilbert Zasoński, **Ewa Stępniak-Konieczna**

Adam Mickiewicz University, Poznań, Poland

Muscleblind-like (MBNL) splicing factors are master regulators of cellular RNA metabolism and homeostasis. Functional depletion of MBNL protein pool caused by nuclear sequestration on CUG repeat-expanded RNA is the main driver of pathological alterations in myotonic dystrophy type 1 (DM1), an inherited neuromuscular disorder. RNA misprocessing and alternative splicing defects (spliceopathy) resulting from loss of functional MBNLs ultimately lead to physio- and histopathological changes within multiple tissues of DM1 patients, particularly skeletal muscle and heart.

In the presented work, we propose novel therapeutic strategy to alleviate the molecular hallmarks of the disease in cellular models of DM1, grounded on transcriptional stimulation of the major mammalian MBNL paralog, MBNL1. In brief, we employed targeted approach for MBNL1 gene upregulation by harnessing the evolutionarily conserved mechanism of RNA activation (RNAa) via small activating RNA duplexes (saRNA) complementary to specific genomic MBNL1 promoter regions. Our preliminary data demonstrates that endogenous modulation of promoter regions via RNAa is a viable option for tuning up MBNL1 gene expression sufficiently enough to enhance transcription, boost cellular MBNL1 protein pool and rescue spliceopathy in DM1 patients derived cellular models, and thus may be used in future therapeutic designs towards DM1.

## Transcription termination in carcinogenesis

**Agata Stępień**, Magda Kopczyńska, Deepshika Pulimamidi, Martyna Plens-Gałąska, Kinga Kamieniarz-Gdula

Center for Advanced Technology and Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Transcription termination is required for proper gene expression. It punctuates the gene ends, releases the engaged RNA Polymerase II (RNAPII), and controls the expression of non-coding RNA. It is coupled with 3' end processing of pre-mRNA catalyzed by cleavage and polyadenylation (CPA) complex. The CPA

factor PCF11 directly binds to the RNAPII C-terminal domain and promotes termination. It can stimulate the full-length termination and premature events that are known regulatory mechanism in yeasts and humans. Since the current understanding of mammalian transcription termination is based on information from highly proliferating and cancer cell lines, we lack knowledge of how transcription termination is shaped in healthy cells and how it changes during transformation.

The aim of our study is to get knowledge about transcription termination during cancer progression and the role of PCF11 in this process. The available data show that in some cancers the levels of PCF11 and other CPA factors are upregulated as compared to the healthy cells. We observed that manipulation of the PCF11 level results in different PCF11 binding to chromatin as wells as transcription termination defects. To further investigate the biology of PCF11 activity we employed the colorectal cancer model in which the level of PCF11 increases with the transformation progression. The PCF11 distribution on chromatin and transcription termination profile will be presented and discussed during the meeting.

## Arabidopsis DXO1 affects the processing of precursors of cytoplasmic and chloroplast ribosomal RNA

**Monika Zakrzewska-Płaczek**, Aleksandra Kwasnik, Michal Krzyszton, Anna Golisz-Mocydlarz, Joanna Kufel

University of Warsaw, Poland

The conserved family of decapping 5'-3' exoribonucleases DXO/Rai1 is involved in mRNA 5’ end quality control, deNADing of non-canonical NAD-capped RNAs and, in yeast, rRNA maturation. Arabidopsis DXO1 (AtDXO1) exhibits plant-specific properties that are reflected in the functions of this enzyme in vivo, e.g. the N-terminal, unstructured extension (NTE), which most likely facilitates interactions with other proteins, but negatively affects biochemical activity.

Our RNA sequencing analyses show that the dxo1 mutation deregulates the expression of many ribosomal protein (RP) genes, which is correlated with inefficient pre-rRNA maturation, both in the nucleolus and chloroplasts. It is possible that the mechanism of regulation of RP gene expression is the result of the cooperation of DXO1 with the mRNA cap methyltransferase RNMT1.

We investigated the role of DXO1 in combination with nuclear XRN2/3 exoribonucleases, known to be involved in rRNA processing. These analyses showed that some of the molecular and morphological phenotypes observed in dxo1 plants were suppressed by XRN3 knockdown, providing evidence for the functional interaction between these proteins.

## Bunyaviral strategies to reorganize and exploit cellular translation

**Piotr Gerlach**

IMol, Polish Academy of Sciences, Warsaw, Poland

Bunyaviruses are a large and understudied group of segmented, negative-sense RNA viruses. Three of them – Lassa, Rift Valley, and Crimean-Congo viruses, causing high fatality rate haemorrhagic fevers, are listed by WHO among eight pathogens that may cause future pandemics. Yet, there is no vaccines or specific antivirals against bunyaviruses. RNA viruses are unconditionally dependent on cellular

protein synthesis machinery. They use a range of strategies to hijack and exploit ribosomes for the translation of their own mRNA. Bunyaviruses have perfected their approach by cap-snatching the cytoplasmic host 5'-capped mRNA and by subsequent coupling of the viral transcription with the ongoing translation. In our newly established lab at IMol, we plan to unravel molecular details of this unique viral translation strategy. We hypothesize that during cap-snatching bunyaviral RNA polymerase hijacks the entire translation initiation complex. This in consequence allows to establish a potentially direct connection between the transcribing viral polymerase and the leading ribosome, unprecedented in the eukaryotic system. Moreover, structured elements within the bunyaviral mRNA UTRs are involved in translation regulation. We have developed a modified orthobunyaviral mini-replicon system in order to identify host factors involved in the bunyaviral translation and to monitor changes in the cellular translation landscape. In parallel, we are going to use cryo-EM to visualize viral-host complexes formed at different steps of the bunyaviral mRNA lifetime. We believe that with such research plan we will open new avenues in the RNA virus field and set the basis for the broad-spectrum antivirals design.

## The transcriptomics of the evolution of insect metamorphosis

**Guillem Ylla**, Gabriela Machaj, Xavier Belles

Laboratory of Bioinformatics and Genome Biology, Jagiellonian University, Kraków, Poland

Insect metamorphosis has fascinated humankind across civilizations, often associated with mysticism and magic. During the 20th century, most of the magical aspects dissipated, but we are still far from understanding how metamorphosis is regulated at the molecular level, and how it emerged and evolved. In the last decade, through the progress in high throughput sequencing technologies, we have been able to study the evolution and regulation of insect metamorphosis by looking at small RNAs, mRNAs, and genomes.

To understand how the complete or holometabolous metamorphosis originated, we compared the transcriptomic changes along the development of insects with this complex development style (e.g. flies & butterflies) with insects with the ancestral incomplete hemimetabolous metamorphosis (e.g. cockroaches & crickets). Among a number of differences, we identified an interesting expression pattern of the transcription factor E93. This transcription factor, known to trigger metamorphosis in the pre-adult stage in all insects, was highly expressed in early embryos of cockroaches but not in flies.

To determine whether this observation was a regularity, we proceed with a comparative analysis of E93 expression across all insect species for which we found publicly available transcriptomes. Our results indicated that E93 expression is high in embryos of insects that do not undergo complete metamorphosis and absent in the embryos of insects that do complete metamorphosis. These results suggest that the loss of expression of E93 in the embryo allowed the emergence of the complete insect metamorphosis, which is currently undergone by 80% of the million described insect species

## Genome-wide identification and classification of sisRNAs in human cells

**Dilek Cansu Gurer**, Merve Kara, İpek Erdoğan-Vatansever, Gökhan Karakülah, Daniel Gautheret, Bünyamin Akgül

Izmir Institute of Technology, Turkey

Introns have been recently recognized as functional units that could exist stably in cells under different conditions despite the historical view of them being unstable sequences that separate exons and to be degraded right after splicing. Stable intronic sequence RNAs (sisRNAs) are a novel type of non-coding RNAs generated through splice-dependent or -independent mechanisms, and are thought to possess a 5' cap and 3' poly-A tails. Studies in X. tropicalis, D. melanogaster, EBV, and mouse have demonstrated that sisRNAs can exist in linear or circular forms, and modulate a variety of cellular functions such as regulation of their host genes, interfering with the small RNA biogenesis pathways, and acting as RNA sponges by base pairing with their target transcripts. Although a couple of intron-derived transcripts have been reported to be functional in human, a genome-wide approach aiming to identify and characterize sisRNA profiles is lacking. Thus, we aimed to investigate and characterize the sisRNA profile in human under drug-induced apoptotic conditions. To this extent, we first performed RNA-seq analyses with apoptotic and non-apoptotic HeLa cells. Since visual and labor-intensive analyses are currently used to find out sisRNAs, we also developed a unique computational tool named sisFindR to facilitate unbiased sisRNA discovery. sisFindR is a pipeline written in Nexflow, which utilizes the k-mer based DE-kupl method and filters the sisRNA candidates using in-house R scripts. The sisRNA candidates identified by sisFindR were then validated by 5' and 3' RACE PCR and characterized functionally.

---

## Structural and biophysical characterization of non-coding RNAs

**Rahul Mehta**, Jakub Nowak, Andrzej Chramiec-Głąbik, Sebastian Glatt

Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Our understanding of the diverse roles of RNAs is growing exponentially. Ribonucleic acids (RNAs) play numerous, distinct and vital roles in biological systems. RNAs, like proteins, have the ability to fold into higher order structures. Nonetheless, structures of proteins represent the vast majority of PDB depositions and less than 1% of available entries describe RNA-only structures. RNA structure determination is complex due to multiple factors and complicated by the inherent ability of RNA domains to adapt multiple conformations. Though, integrative structural biology can help to overcome these problems. Most studies related to RNA structures have focused on the structure determination of enzymatically active ribozymes or riboswitches. RNA domains in non-catalytic RNAs fold into largely unknown three-dimensional structures. Although sequence information and folding predictions for structured RNAs is widely available, efficient structural determination lags behind, hindering their holistic molecular characterization. Our collaborative project is aimed at the structural determination of RNA molecules using single particle cryo-EM combined with approaches such as biochemical screening using biophysics. In detail, we develop novel biophysical techniques to determine optimal folding conditions for numerous target RNAs that are subsequently analysed using cryo-EM. The experimentally determined maps are interpreted using computational modelling of the respective RNA molecules, leading to three-dimensional pseudo-atomic models of the respective RNAs. The ultimate

aim of the project is to establish high-throughput structure determination pipelines that are generically applicable for all types of RNA molecules. Our early results indicate a high success rate, leading to the de novo determination of numerous scaffold RNAs from different organisms. Our work aims to narrow the wide gap between vast sequence information and minuscule three-dimensional structures of RNA molecules.

## Structural studies of the Betacoronaviruses 5'-proximal regions

Agata Bernat[1#], Elżbieta Purta[1#], **Tales Rocha de Moura**[1#], Małgorzata Kurkowska[1], Eugene F. Baulin[1], Sunandan Mukherjee[1], Eva M. Martin-Cuevas[2], Jakub Nowak[3], Artur P. Biela[4], Michal Rawski[3], Sebastian Glatt[3], Fernando Moreno Herrero[2], Janusz M. Bujnicki[1#]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Department of Macromolecular Structures, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain
[3] Max Planck Research Group at the Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland
[4] Bionanoscience and Biochemistry Laboratory at the Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland
[#] equal contribution

The emergence of the COVID-19 pandemic has revealed an urgent need for better understanding of the molecular mechanisms of SARS-CoV-2 function, which can guide the development of diagnostic and antiviral strategies targeting the virus. SARS-CoV-2 is a member of Coronaviridae (CoVs), a group of evolutionary related single-stranded, non-segmented, enveloped RNA viruses, which cause respiratory, enteric, hepatic and neurological diseases in a broad range of vertebrate species. Most human coronaviruses commonly cause relatively mild respiratory disease; however several coronaviruses including SARS-CoV, MERS-CoV, and now SARS-CoV-2 can cause severe illness and death. The above-mentioned coronaviruses belong to the Betacoronavirus (βCoV) genus, qualifying it as the main clinical threat to humans. Betacoronavirus is one of four genera of coronaviruses, and it consists of A, B, C and D lineages. To comprehend the threat posed by βCoV to humans, it is essential to grasp the molecular diversity of these viruses, encompassing both shared components and variations. With the assistance of chemical probing, our group had previously identified several folded RNA structures in the SARS-CoV-2 genome. In the presented work, we focus on the 5' untranslated region (UTR) from representatives of the four βCoV lineages, including SARS-CoV-2 (representing the B lineage). Chemical probing results enabled us to identify stable fragments of the 5' UTRs suitable to structural studies by Cryo-electron microscopy (Cryo-EM). The main identified fragment was the 5' SL5 and we were able to obtain Cryo-EM maps from the four βCoV lineages representatives: (A) OC43-CoV, (B) SARS-CoV-2, (C) MERS-CoV, and (D) Rousettus-CoV. All the reconstituted maps reached mid-range resolution around 7 Å. Using a computational method developed in-house (SimRNA-cry, unpublished) we were able to obtain atomic models using the chemical probing and Cryo-EM results.

## Intracellular damage of mcm5S2U-tRNA induced by oxidative stress

**Małgorzata Sierant**, Rafal Szewczyk, Agnieszka Dziergowska, Barbara Nawrot

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland

Transfer RNAs play an extremely important function in every cell, which is to accurately translation of the genetic code in messenger RNA into the amino acid sequence of a newly synthesized protein. We have previously shown that 2-thiouridine (S2U), either as a single nucleoside or as a component of a model RNA oligonucleotide chain, is effectively desulfured under the influence of oxidative reagents 1,2. Chemically induced desulfuration of S2U results in two products: 4-pyrimidinone nucleoside (H2U) and uridine (U). We are currently investigating whether desulfuration of S2U is a natural process that occurs in cells exposed to oxidative stress or whether it occurs only in the test tube during chemical reaction with oxidants at high concentrations. Experiments with yeast and human cells exposed to H2O2 confirmed that 5-substituted 2-thiouridines present exclusively in the wobble position (position 34) of the anticodon of some tRNAs are oxidatively desulfured. The LC-MS/MS quantitative analysis of the nucleoside mixtures obtained after tRNA hydrolysis revealed the presence of mcm5H2U and mcm5U products of desulfuration of the parent mcm5S2U. We also observed immature cm5S2U, cm5H2U, and cm5U products, which may indicate a disruption of the enzymatic modification process at the C5 position of 2-thiouridine. The observed process triggered by oxidative stress in living cells could affect the function of 2-thiouridine-containing tRNAs and alter the translation of genetic information.

---

## Cas12a2 nucleases form three functionally-distinct clades

**Oleg Dmytrenko**[1], Xiye Chen[1], Ryan Jackson[2], Chase Beisel[1]

[1] Helmholtz HIRI, Würzburg, Germany
[2] Utah State University, Logan, UT, USA

Cas12a2 is a novel group of CRISPR-Cas nucleases. We have previously characterized a Cas12a2 ortholog from Sulfuricurvum sp., an RNA-targeting nuclease with collateral activity towards dsDNA, ssDNA, and RNA. We demonstrated that SuCas12a2 can enact population-level immunity by degrading non-targeted nucleic acids, including chromosomal DNA, and, therefore, eliminating the infected bacteria once the infectious target is recognized. Since then, we identified over seventy Cas12a2 orthologs that form three distinct phylogenetic clades: Su, Sm, and Ap. Orthologs within each clade share unique amino acid motifs and predicted structural domains, while shared amino acid identity between the clades can be as low as 8%. For instance, the Sm and Ap clade orthologs lack many of the residues found in SuCas12a2, such as the aromatic amino acids required for collateral DNA cleavage. Based on these differences, we hypothesize that Cas12a2 orthologs from the three clades are functionally distinct, especially with regard to their collateral activity. To investigate this diversity, we first identified a set of active Cas12a2 nucleases from each clade. Next, we demonstrated that these representative orthologs all target RNA. Despite this commonality, the orthologs showed distinct clade-specific preference for collateral substates. They further greatly differed in their preference for protospacer flanking sequences (PFS) and in metal ion requirements. Further characterization of Cas12a2 orthologs from the three clades is ongoing. This work highlights the previously unknown functional diversity of Cas12a2 nucleases and informs their potential downstream applications.

# Posters

## Poster Session I

| Poster No | Authors | Title |
|---|---|---|
| 1 | **Klaudia Samorowska**, Elżbieta Wanowska, Michał Szcześniak | Long noncoding RNAs in breast cancer epitranscriptomics |
| 2 | **Jakub Dolata**, Agata Stępień, Dawid Bielewicz, Tomasz Gulanicz, Mateusz Bajczyk, Łukasz Szewc, Monika Jóźwiak, Dariusz J. Smoliński, Zofia Szweykowska-Kulińska, Artur Jarmołowski | Co-transcriptional miRNA biogenesis in plants |
| 3 | **Joanna Kwiatkowska**, Ewa Stein, Anastasiia Romanenko, Uwe Kuhn, Elmar Wahle, Kinga Kamieniarz-Gdula | In vitro reconstitution of pre-mRNA 3'-end processing machinery |
| 4 | **Ramasamy Subbiah** | Can Xenobiotics induce RNA Modifications as diagnostic and therapeutic approaches for cardiac ailments? |
| 5 | Patrycja Plewka, Robert Pasieka, Michał W. Szcześniak, Elżbieta Wanowska, Izabela Makałowska, **Dorota Raczyńska** | Novel role of U7 snRNA in the negative regulation of lincRNA and HERVs expression in human cells |
| 6 | **Gabriela Betlej**, Iwona Rzeszutek, Anna Lewińska, Maciej Wnuk | TRDMT1 methyltransferase affects extracellular RNA-based responses in etoposide-stimulated senescent osteosarcoma cells |
| 7 | **Paulina Glazińska**, Wojciech Glinkowski, Milena Kulasek | Evolutionary conserved and novel lupine-specific regulation of ARF6 gene expression by miRNA and phased ta-siRNA |
| 8 | **Dominika Błoniarz**, Jagoda Adamczyk-Grochala, Anna Lewińska, Maciej Wnuk | Changes in telomere length and related mechanisms in TRDMT1 gene knockout cancer cells during long-term selection upon azacytidine stimulation |
| 9 | **Aneta Łyś**, Natalia Ryczek, Izabela Makałowska | The functional meaning of 5'-end-extended transcripts of protein-coding genes in human |
| 10 | **Upasana Saha**, Lee Davidson, Steven West, Elmar Wahle, Kinga Kamieniarz-Gdula | Mechanistic dissection of human PCF11 in premature and normal transcription termination |
| 11 | **Julia Kurzawska**, Yeva Kovalyk, Maciej Basczok, Ewa Stein, Mikołaj Olejniczak | RNA binding by KH-domain proteins KhpA and KhpB from Streptococcus pneumoniae |
| 12 | **Jadwiga Meissner** and Magda Konarska | The function of 5' exon motifs in stabilizing the 5'SS within the yeast spliceosome |

| | | |
|---|---|---|
| **13** | **Bartosz Tarkowski**, Hanna Grzesik, Wiktoria Orzeł, Paweł Krawczyk, Karolina Piechna, Jan Brancewicz, Olga Gewartowska, Kamil Jachacy, Jakub Gruchota, Marcin Szpila, Katarzyna Sałas, Sara Domagała, Monika Pawłowska, Andrzej Dziembowski | Cytoplasmic poly(A) polymerase TENT5A is a global positive regulator of neurohormone expression |
| **14** | **Bharti Aggarwal**, Halina Pietrykowska, Wojciech M. Karłowski, Artur Jarmołowski, Zofia Szweykowska-Kulińska | Functional characterization of liverwort-specific and Marchantia-specific miRNAs |
| **15** | **Szymon Krasnodębski**, Agnieszka Gozdek, Maria-Bianca Mititelu, Oldřich Hudeček, Hana Cahova, Joanna Kufel | Arabidopsis thaliana NudiXes |
| **16** | **Jingping Geng**, Magdalena Chrabąszczewska, Karol Kurpiejewski, Marzena Jankowska-Anyszka, Anna Stankiewicz-Drogon, Edward Darzynkiewicz, Renata Grzela | Kinetic analysis of IFITs interactions and the role of IFITs complexes in IFIT1 binding to IVT mRNA |
| **17** | **Milena Krajewska**, Zuzanna Napora, Edward Darżynkiewicz, Renata Grzela | Potential interaction of PNRC1 protein with proteins forming the decapping complex |
| **18** | **Emilia Kozłowska**, **Agata Ciołak**, Grażyna Adamek, Julia Szcześniak, Agnieszka Fiszer | microRNA-mediated dysregulation of transcription in Huntington's disease |
| **19** | **Anna Golisz-Mocydlarz**, Monika Zakrzewska-Płaczek, Michał Krzysztoń, Justyna Piotrowska, Joanna Kufel | NOL12 is required for pre-rRNA processing and regulates this process in response to stress in Arabidopsis |
| **20** | **Jakub Piątkowski** and Paweł Golik | A new approach to studying Candida albicans mitochondrial transcriptome via direct RNA sequencing |
| **21** | **Michał Brouze**, Monika Kusio-Kobiałka, Olga Gewartowska, Agnieszka Czarnocka-Cieciura, Marcin Szpila, Seweryn Mroczek, Bartosz Tarkowski, Kamil Jachacy, Andrzej Dziembowski | TENT5-mediated cytoplasmic polyadenylation of mRNAs encoding secreted proteins is essential for oogenesis in mice |
| **22** | **Anna Stankiewicz-Drogon**, Renata Grzela, Siranjeevi Nagaraj, Anna Andrzejewska, Piotr Rogujski, Edward Darzynkiewicz, Barbara Łukomska, Mirosław Janowski | hBM-MSCs engineering with ARCA-capped mRNA |
| **23** | **Magdalena Wrona**, Julia Zinsmeister, Ruslan Yatusevich, Szymon Świeżewski | A long non-coding antisense transcript of DOG1 regulates Arabidopsis thaliana secondary seed dormancy by BRM, a SWI/SNF ATPase |
| **24** | **Maciej M. Basczok** and Mikołaj Olejniczak | FinO-domain protein ProQ from Neisseria meningitidis recognizes intrinsic transcription terminators in its RNA targets |
| **25** | **Martyna Koźbiał**, Helena Kossowska, Marta Gapińska, Roksana Iwanicka-Nowicka, Wojciech Wróblewski, Marta Góra, Michał Krzysztoń, Szymon Świeżewski, Małgorzata Lichocka, Antoni Palusiński, Katarzyna Sosnowska, Marta Koblowska | Arabidopsis homolog of human CWC22 is required for seed development |

| | | |
|---|---|---|
| **26** | Vladyslava Liudkovska, Kevork Wakimian, Ashwini Khaladkar, Phuong Cao Thi Ngoc, Cristian Bellodi, **Maciej Cieśla** | Splicing factor dynamics in steady-state and malignant hematopoiesis |
| **27** | **Anna Pacwa**, Joanna Machowicz, Piotr Rodak, Bartosz Machna, Saeed Akhtar, Marialaura Amadio, Joanna Lewin-Kowalik, Adrian Smędowski | HuR silencing promotes Retinal Ganglion Cells degeneration and alleviates the activity of exogenous neuroprotection in glaucoma |
| **28** | **Magda Kopczyńska**, Chihiro Nakayama, Agata Stępień, Takayuki Nojima, Kinga Kamieniarz-Gdula | Genome-wide analysis of chromatin signature/composition at human transcription termination regions |
| **29** | **Małgorzata Zawadzka**, Leszek Błaszczyk, Urh Černigoj, Fayza Daboussi, Mara G. Freire, Julita Gumna, Katarzyna Pachulska-Wieczorek, Mario Perkovic, Rok Sekirnik, Fani Sousa, Chantal Pichon | Yeast-based factory for therapeutic mRNA bioproduction |
| **30** | **Maria Jagielska**, Paweł Hałakuc, Rafał Milanowski | Nonconventional introns as factors affecting the coding potential of euglenid genes |
| **31** | **Marta Starczak**, Fabian Leśniewski, Maciej Gawroński, Daniel Gackowski | Detection and quantification of RNA modifications using UPLC-MS/MS |
| **32** | **Moh Egy Rahman Firdaus**, Eliana Dukhno, Piotr Gerlach | Identification of cellular factors associated with the bunyaviral transcription and translation |
| **33** | **Dominika Maziarz**, Karolina Sapoń, Teresa Janas, Tadeusz Janas | Selection of miRNAs specific to exosomal membranes |
| **34** | **Magdalena Jazurek-Ciesiołka**, Adam Ciesiołka, Emilia Kozłowska, Grzegorz Figura, Michał W. Szcześniak, Edyta Kościańska, Paweł Joachimiak, Grażyna Adamek, Izabela Makałowska, Agnieszka Fiszer | New insights into the complexity of SCA3: the contribution of non-coding RNAs |
| **35** | **Iga Gawrońska**, Karolina Sapoń, Teresa Janas, Tadeusz Janas | Effect of polysialic acid and galacturonic acid on RNA binding to exosomal membrane |

**1**

## Long noncoding RNAs in breast cancer epitranscriptomics

**Klaudia Samorowska**, Elżbieta Wanowska, Michał Szcześniak

Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Natural antisense transcripts (NATs) are a fraction of long noncoding RNA (lncRNAs), that are transcribed from DNA strands opposite to other genes normally being protein-coding. They can play numerous functions which depends on their cellular localization. NATs located in nucleus, for example, may interact with their sense partners and make double stranded structures called RNA:RNA duplexes. Those structures are required for enzymes conducting A-to-I RNA editing process. ADAR (Adenosine Deaminase Acting on RNA) enzymes recognize RNA:RNA duplexes and convert adenines to inosines (A-to-I). During translation, inosines are read as guanosines, which has functional implications very similar to genetic mutations. Process of A-to-I RNA editing may influence progression of various cancers, including breast cancer, one of the most prevalent malignancies.

In our research, we look for evidence that NATs might be a factor which triggers A-to-I RNA editing in breast cancer. We identified candidate transcripts that may be related to this event using in silico studies. We have verified the presence of RNA editing in breast cancer cell lines using Sanger sequencing of mRNAs and associated DNA sequences. In addition, we discovered that the NATs of interest may interact with their protein coding partners and create RNA:RNA duplexes. Now, we are working on knocking down the studied lncRNAs, to investigate their roles in RNA editing status and impact on biology of breast cancer cells.

**2**

## Co-transcriptional miRNA biogenesis in plants

**Jakub Dolata**, Agata Stępień, Dawid Bielewicz, Tomasz Gulanicz, Mateusz Bajczyk, Łukasz Szewc, Monika Jóźwiak, Dariusz J. Smoliński, Zofia Szweykowska-Kulińska, Artur Jarmołowski

Adam Mickiewicz University, Poznań, Poland

The maturation of nascent RNA is tightly connected with the transcription process. It is well known for animals and plants that RNA polymerase II (RNAPII) elongation rate, pausing, and termination steps may affect the quality and quantity of its final product: mature mRNA. Pre-mRNA splicing, incorporation of RNA modifications as well as 3' end maturation are co-transcriptional. However, for years plant miRNA biogenesis was considered post-transcriptional and localized in so-called D-bodies (Dicing boodies) in the cell nucleus. Our studies clearly show that in Arabidopsis, miRNA production takes plays already during transcription and it is tightly regulated at multiple levels. Moreover, protein factors known for their direct involvement in the miRNA pathway are also important for the transcription and maturation of pre-mRNA.

**3**

## In vitro reconstitution of pre-mRNA 3'-end processing machinery

**Joanna Kwiatkowska**[1], Ewa Stein[1], Anastasiia Romanenko[1], Uwe Kuhn[2], Elmar Wahle[2], Kinga Kamieniarz-Gdula[1]

[1] Institute of Molecular Biology and Biotechnology, Faculty of Biology and Center for Advanced Technology, Adam Mickiewicz University, Poznań, Poland

[2] Institute of Biochemistry and Biotechnology, Martin Luther University of Halle-Wittenberg, Halle, Germany

The complexity of living organisms depends on transcriptome regulation and expression. Inaccuracy of these processes can cause developmental aberrations, or diseases. Several regulatory mechanisms are used by the cells to ensure transcription accuracy, and to exploit the mRNA coding potential. Alternative splicing can result in multiple protein isoforms. Importantly, localization of cleavage and polyadenylation (CPA) within precursor mRNAs can also generate transcript isoforms with different 3' ends. This requires endonucleolytic cleavage of pre-mRNA and polyadenylation carried out by cleavage and polyadenylation specificity factor (CPSF), cleavage factors I and II (CF I and II), cleavage stimulation factor (CstF), poly(A) polymerase, and at least in vitro, Rbbp6.

Our lab is interested in the mechanism of 3' end selection. We would like to elucidate the intrinsic sequence specificity of the cleavage machinery. In the first step we are planning to reconstituted the 3' end processing machinery. For this purpose we perform synthesis of CPA proteins in bacteria and in Sf21 insect cells/baculovirus system followed by recombinant proteins purification. Obtained proteins will be checked in in vitro pre-mRNA cleavage assay. As radiolabeled RNA substrates we will use viral sequences. To expand our view on 3' ends selection we will use endogenous human RNAs with single or double polyadenylation sites.

In summary, in the first part of the project we aimed to: in vitro reconstitute the 3'end processing machinery, test its potential for cleavage of RNA substrates, understand the selection of polyadenylation sites. These general steps allow us to better understand how 3'ends are formed.

**4**

## Can Xenobiotics induce RNA Modifications as diagnostic and therapeutic approaches for cardiac ailments?

**Ramasamy Subbiah**

Cardiac Metabolic Disease Laboratory, Department of Biochemistry, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nādu, India

Post-transcriptional RNA modification has been observed throughout the kingdoms of life and more than many different types of RNA modifications dominate the chemical and structural properties of these nucleotides. The RNA modifications can potentially alter the RNA topology and affect the binding affinity of proteins, thus regulating the mRNA stability as well as translation. Promising evidence suggest that these modifications are not stable, but dynamic; vary upon different cues and specific to tissue types. The cardiac transcriptome is not exceptional to such RNA modifications and is enriched with the abundant base methylation such as N6-methyladenosine (m6A) and also 2'-O-Methylation (Nm). In the present study, we focused on RNA modifications in CVDs (cardiovascular diseases) and the interplay between RNA modifications and microRNAs (small noncoding RNAs). The study suggested that microRNAs specific to xenobiotics (monocrotophos and chlorpyrifos) are the key player in RNA modifications induced cardiac manifestations.

---

**5**

## Novel role of U7 snRNA in the negative regulation of lincRNA and HERVs expression in human cells

Patrycja Plewka[1], Robert Pasieka[1], Michał W. Szcześniak[2], Elżbieta Wanowska[2], Izabela Makałowska[2], **Dorota Raczyńska**[1]

[1] Department of Gene Expression, Laboratory of RNA Processing, Institute of Molecular Biology and Biotechnology, Faculty of Biology and Center for Advanced Technology, Adam Mickiewicz University, Poznań, Poland

[2] Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

U7 snRNA is a part of U7 snRNP, a ribonucleoprotein complex required for the 3'end processing of replication-dependent histone pre-mRNAs in the replication phase. During this maturation event, the 5’ region of U7 snRNA hybridizes with the highly complementary sequence present in the 3’UTR of histone pre-mRNAs, called histone downstream element, HDE. This base pair interaction triggers subsequent reactions that eventually result in cleavage and releasing of mature histone transcripts. Interestingly, although its function is tightly restricted to S phase, the complex is constitutively expressed throughout the cell cycle and in nondividing cells, suggesting another function in cells.

Recently, we have performed high-throughput sequencing of RNA isolated from cells with U7 snRNA knockdown and found that several long intergenic noncoding RNAs (lincRNAs) and transposon elements of human endogenous retroviruses (HERVs) class were significantly upregulated. Both U7-dependent lincRNAs and HERVs contain HDE-like motifs that perfectly match the 5' end of U7 snRNA and can form base pair-interacting platforms. Indeed, mutations within these motifs abrogate U7 snRNA regulatory function and stimulate the expression of lincRNAs and HERVs. Furthermore, in the cell model of amyotrophic lateral sclerosis with disrupted U7 snRNA localization (Gadgil et al., Scientific Rep. 2021) we also observed upregulated expression of U7-dependent lincRNAs and HERVs, suggesting their role in neurodegeneration.

Here, we will propose the mechanism of transcription inhibition by which U7 snRNA plays a protective role in keeping these deleterious genetic elements in silence.

This work was supported by the National Science Centre (UMO-2018/30/E/NZ2/00295).

**6**

## TRDMT1 methyltransferase affects extracellular RNA-based responses in etoposide-stimulated senescent osteosarcoma cells

**Gabriela Betlej**, Iwona Rzeszutek, Anna Lewińska, Maciej Wnuk

University of Rzeszów, Poland

Nucleic acids released from dying or senescent cells can be considered as damage-associated molecular patterns that may activate innate immune-like responses and act as a double-edged sword in anticancer therapies. TRDMT1 methyltransferase, involved in DNA damage and pro-inflammatory responses, is suggested to be a new target in anticancer therapies. The data on extracellular RNA-associated effects in cancer cells and their therapeutic implications are limited. In the present study, we have asked if the treatment with extracellular RNA may be modulated by the activity of TRDMT1 methyltransferase in cancer cells. To do so, three cellular models of osteosarcoma were used with CRIPSR/Cas9-based TRDMT1 gene knockout (KO). Cells were stimulated with RNA released from dying or senescent cancer cells using death- or senescence-inducing concentrations of etoposide. The effects of extracellular RNA were investigated in non-senescent and senescent osteosarcoma cells using chemotherapy-induced model of cancer cell senescence. The activities of nucleic acid sensing pathways and inflammatory markers were then evaluated using single cell-based approach and cytometric measurements. Etoposide-induced senescent cancer cells were susceptible to the treatment with extracellular RNA and in the case of U-2 OS cell line, TRDMT1 KO potentiated RNA-mediated cytotoxic effect that was mediated by IL-8 secretion. The activity of STING and the levels of APOBEC3A, NSUN3 and NSUN5 were also stimulated in RNA-treated senescent osteosarcoma cells and TRDMT1 KO attenuated this effect. TRDMT1 is suggested to be a regulator of extracellular RNA-induced responses in chemotherapy-associated senescent cancer cells.

This study was supported by National Science Centre (Poland) grant UMO-2021/43/B/NZ2/02210.

**7**

## Evolutionary conserved and novel lupine-specific regulation of ARF6 gene expression by miRNA and phased ta-siRNA

**Paulina Glazińska**[1,3], Wojciech Glinkowski[1,3], Milena Kulasek[1,2,3]

[1] Department of Plant Physiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Poland

[2] Department of Genetics, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Poland

Using next-generation sequencing (NGS) of sRNA, transcriptomes and degradome libraries, we identified miRNA, phased ta-siRNAs, lncRNA, and their target genes in yellow lupine (Lupinus luteus L.) developing pods. Among the identified miRNA and siRNAs, we find that two sRNAs, namely miR167 and Ll-ta-siR2, might target LlARF6 sequences (unigene TRINITY_DN50857_c0_g2). In other plant species, ARF6 is regulated by miR167 canonically. The regulation of LlARF6 transcripts by Ll-ta-siR2 in pods of L. luteus discovered by our team is the first reported interaction of this kind. Our bioinformatic analyses suggest that Ll-ta-siR2 might originate from duplicated LlARF6 gene inserted into the yellow lupine genome in the reverse orientation. To determine if this situation occurs only in yellow lupine or is common in another lupine species, we analysed genomes of the closest related species L. albus and L. angustifolius.

Furthermore, the deeper analyses of LlARF6 sequences showed that it could be a source of another ta-siRNA, namely Ll-ta-siR27, which targets transcript encoding putative pre-16S rRNA nuclease. We hypothesise that the cleavage of LlARF6 transcript by miR167 or by Ll-ta-siR2 could be a source for Ll-ta-siR27.

The presented data shed new light on the molecular network regulating the development of pods in lupines.

---

**8**

## Changes in telomere length and related mechanisms in TRDMT1 gene knockout cancer cells during long-term selection upon azacytidine stimulation

**Dominika Błoniarz**, Jagoda Adamczyk-Grochala, Anna Lewińska, Maciej Wnuk

University of Rzeszów, Poland

TRDMT1 (former name DNMT2), 5-methylcytosine RNA methyltransferase is implicated in the regulation of cell proliferation, senescence and stress responses such as DNA damage response in normal and cancer cells. However, little is known about TRDMT1-mediated changes in genomic stability and their consequences in cancer cells subjected to anticancer therapy. In the present study, four

cellular models of cancer (cervical with wild type TERT, breast with mutated TERT, osteosarcoma with no telomerase activity, glioma with mutated TERT) were used to evaluate the effects of TRDMT1 gene knockout (KO) on telomere length during long-term culture in the presence of an anticancer drug azacytidine. Observed changes in telomere length were further confronted with the expression and protein levels of regulators of telomere maintenance such as shelterin complex, TERRA expression and telomerase activity. The interactions between TRDMT1 and TERT, a protein subunit of telomerase, were also studied. Obtained results are presented and discussed in the context of TRDMT1-mediated modulation of genetic, here telomeric sequences, plasticity of cancer cells during drug treatment and selection and its implications for anticancer therapy.

This study was supported by the National Science Centre (grant no. 2017/25/B/NZ2/01983).

**9**

## The functional meaning of 5'-end-extended transcripts of protein-coding genes in human

**Aneta Łyś**, Natalia Ryczek, Izabela Makałowska

Adam Mickiewicz University, Poznań, Poland

The utilization of more distal alternative transcription start sites leads to the formation of transcripts extended at their 5' ends. In result, the overlap with the gene located on the opposite DNA strand may occur. This genomic arrangement may have negative impact on the expression level of involved genes due to e.g. transcriptional interference, promoter competition, or RNA:RNA duplex formation. Despite some research on the subject, biological meaning of both, an extension of the transcripts at their 5' ends and the overlap between two protein-coding genes have not been fully understood yet.

We performed studies aiming at deciphering the role of 5' extended transcripts that overlap a gene on the opposite DNA strand. Two pairs of human overlapping protein-coding genes, INO80E:HIRIP3 and CENPL:DARS2, were closely investigated. Results of performed experiments demonstrate that extended transcripts INO80E-201 and CENPL-201, form RNA:RNA duplexes with transcripts of HIRIP3 and DARS2 respectively. In addition, both elongated at 5' end transcripts had a very low expression level and were located almost exclusively in the nucleus. Interestingly, silencing of these transcripts led to the significant decrease in the expression of both genes in each pair. However, silencing of genes on opposite DNA strand, HIRIP3 and DARS2, had different effect, the expression of INO80E and CENPL increased. Results of our study strongly demonstrate that overlapping transcripts of protein-coding genes' have significant regulatory role and are required to secure higher expression level of involved in the overlap genes.

**10**

## Mechanistic dissection of human PCF11 in premature and normal transcription termination

**Upasana Saha**[1], Lee Davidson[2], Steven West[2], Elmar Wahle[3], Kinga Kamieniarz-Gdula[1]

[1] Center for Advanced Technology and Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

[2] The Living Systems Institute, University of Exeter, UK

[3] Institute of Biochemistry and Biotechnology, Charles Tanford Protein Center, Martin Luther University Halle-Wittenberg, Halle, Germany

Transcription by RNA Polymerase II stands at the heart of the 'Central Dogma of Molecular Biology' ensuring the unidirectional flow of genetic information from DNA to RNA. Although transcription has been studied for decades, our understanding of transcription termination and 3'-end processing remains limited. Through this work we wish to shed light on the cross-talk between these closely connected yet mutually independent regulatory events shaping the 3'-end of genes.

PCF11, an essential autoregulatory protein and an integral component of mammalian cleavage factor II (CF IIm), has been known to be involved in multiple functions along the RNA biogenesis pathway. It plays an important role in transcription termination and is a key player in cleavage and polyadenylation. Yet another newly discovered function of PCF11 is its ability to prematurely terminate a subset of genes in human cells and during zebrafish development. The genes are mainly enriched for gene expression regulators.

This project aims to understand the ability of each of the domain/domains to trigger normal and premature transcription termination in mammalian cells. By separating out the domains of PCFII we hope to understand how each of these domains influence the various known functions of PCFII from a physiological standpoint. A wider scope of the project is to investigate and understand the mechanism and regulatory potential of premature transcription termination in vertebrates which remains yet to be explored.

## 11

## RNA binding by KH-domain proteins KhpA and KhpB from Streptococcus pneumoniae

**Julia Kurzawska**, Yeva Kovalyk, Maciej Basczok, Ewa Stein, Mikołaj Olejniczak

Adam Mickiewicz University, Poznań, Poland

The functions of bacterial small regulatory RNAs (sRNAs) are often dependent on RNA-binding proteins. In many Gram-negative bacteria sRNAs are assisted by the chaperone protein Hfq, and by FinO-domain proteins. On the other hand, in Gram-positive bacteria the functions of sRNAs and their cooperation with RNA-binding proteins are much less well known. Recently, it has been reported that KH domain-containing proteins KhpA and KhpB bind numerous RNAs in Streptococcus pneumoniae and other Gram-positive bacteria. Additionally, both proteins are involved in cell division. KhpA consists of a single KH domain, and KhpB contains two RNA-binding domains, KH and R3H, and a Jag-N domain of unknown function. While the RNAs bound by these proteins have been identified, it is not known how they recognize their RNA ligands.

To explore how KhpA/B proteins bind RNAs, we cloned, overexpressed in E. coli cells, and purified KhpA protein, and a fragment of KhpB protein consisting of KH and R3H domains. We also used a gelshift assay to analyze the pairing interactions between one of the RNA ligands of these proteins, CcnD sRNA, and two mRNAs that it regulates. Next, we plan to analyze the kinetic and thermodynamic framework of KhpA/B interactions with CcnD sRNA and regulated mRNAs to better understand how KhpA/B proteins recognize their RNA targets.

---

## 12

## The function of 5' exon motifs in stabilizing the 5'SS within the yeast spliceosome

**Jadwiga Meissner** and Magda Konarska

IMol, Polish Academy of Sciences, Warsaw, Poland

To better understand the role of exons in splicing, we identified 5' exon motifs that improve splicing of suboptimal 5'SS-G5a introns in S. cerevisiae. Selected sequences form two sub-motifs: A-rich and C-rich, that act additively, indicating that they affect two distinct events during splicing.

Our results suggest that A-rich motifs stabilize the 5'SS binding to U1. We consider the Luc7 N-terminal domain as a binding site for A-rich motifs. To test this hypothesis, we analyze deletions of the Luc7 N-terminal domain in the context of reporters containing A-rich motifs and a G5a mutation.

The C-rich motifs improve splicing of introns limiting for the first step but inhibit splicing of introns with the second step defects. We suggest that C-rich motifs stabilize 5'SS positioning for the first step of catalysis. The observed inhibition of the first-to-second step transition indicates that stable first-step interactions must be destabilized between the two steps.

A-rich motifs are widespread among yeast exons, suggesting their role in splicing of endogenous genes. C-rich motifs are found in 29 exons, 12 of which are two-intron or alternatively spliced genes, suggesting a regulatory role of C-rich motifs in splicing. We are analyzing the effects of mutations of C-rich motifs on splicing of selected endogenous genes, with a particular interest in the PTC7 gene product, which is functional in both spliced and unspliced isoforms. Understanding functional exon interactions will help formulate a general mechanism of splicing enhancement by regulatory exon motifs acting within the spliceosome.

---

**13**

## Cytoplasmic poly(A) polymerase TENT5A is a global positive regulator of neurohormone expression

**Bartosz Tarkowski**[1], Hanna Grzesik[1], Wiktoria Orzeł[1], Paweł Krawczyk[1], Karolina Piechna[1], Jan Brancewicz[1], Olga Gewartowska[1], Kamil Jachacy[1], Jakub Gruchota[1], Marcin Szpila[1], Katarzyna Sałas[1], Sara Domagała[1], Monika Pawłowska[2], Andrzej Dziembowski[1]

[1] International Institute of Molecular and Cell Biology in Warsaw, Poland

[2] Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Specialized secretory neurons in the hypothalamus and the Edinger-Westphal nucleus secrete several neurohormones. Very little is known about the regulation of their expression at the post-transcriptional level. Here we discovered that a non-canonical cytoplasmic poly(A) polymerase Tent5a is a global positive regulator of neurohormone expression. We detect Tent5a in Edinger-Westphal, paraventricular and supraoptic nuclei, as well as the lateral hypothalamic area. Importantly, RNA sequencing of these regions' biopsies, as well as northern analysis of the whole hypothalami, revealed decreased expression of several neuropeptides mRNA in tent5a knockout mice, like vasopressin, oxytocin, urocortin, and CART peptide. In contrast, corticotropin-releasing factor expression, for instance, remained unchanged both on mRNA and protein levels, indicating selectivity of Tent5a targets. To check if Tent5a directly polyadenylates downregulated mRNAs, we performed genome-wide poly(A) tail length profiling of RNA from the hypothalamus on the Oxford Nanopore platform. Such analysis revealed that mRNAs encoding vasopressin and oxytocin have greatly reduced poly(A) tail length (from ~200 to ~100bp), which is not restored to the physiological levels upon dehydration. Our results indicate a novel mechanism of ensuring efficient baseline neuropeptide secretion by stabilizing their mRNAs with Tent5a-mediated polyadenylation in the cytoplasm.

---

## 14

## Functional characterization of liverwort-specific and Marchantia-specific miRNAs

**Bharti Aggarwal**, Halina Pietrykowska, Wojciech M. Karłowski, Artur Jarmołowski, Zofia Szweykowska-Kulińska

Adam Mickiewicz University, Poznań, Poland

MicroRNAs (miRNAs) have a significant impact on the plant growth and developmental processes because they are the crucial post-transcriptional switches of eukaryotic gene expression. Functional studies on the role of conserved miRNAs in land plants have been published using model organisms like Arabidopsis thaliana and Physcomitrium patens. However, plants use species-specific miRNAs to control a vast array of biological processes. Therefore, as Marchantia polymorpha occupies a significant phylogenetic position in land plant evolution for evo-devo research, we seek to elucidate the function of liverwort-specific and Marchantia-specific miRNAs in the developmental pathways. Three liverwort-specific miRNAs (Mpo-miR11737a, Mpo-miR8170, Mpo-miR8166) and two Marchantia-specific miRNAs (Mpo-miR11796 and Mpo-miR11887) were identified as putative players in the sexual reproduction of Marchantia. These miRNAs exhibited differential expression in Marchantia vegetative and generative thalli. Mpo-miR11737a is upregulated in the male and female vegetative thalli, Mpo-miR8170 is upregulated in the female archegoniophores as well as male antheridiophores and Mpo-miR8166 is highly expressed in male antheridiophores. Mpo-miR11796 is abundantly accumulated in the female archegoniophores and Mpo-miR11887 has exceptionally high expression specifically in the antheridiophores. We first defined the gene structure of these MIR genes in order to elucidate their transcriptional units and comprehend their processing due to their intriguing expression pattern. Additionally, we constructed the CRISPR-Cas9 KO mutant lines for these miRNAs and analyzed the phenotype of mutant plants for their ability to grow and produce gametangiophores.

## 15

## Arabidopsis thaliana NudiXes

**Szymon Krasnodębski**, Agnieszka Gozdek, Maria-Bianca Mititelu, Oldřich Hudeček, Hana Cahova, Joanna Kufel

Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland

Recent discoveries have proven that non-canonically capped transcripts (e.g. NAD-RNA, NpnN) are present in both prokaryotic and eukaryotic cells, although their metabolic functions still remain unclear. NAD-capped transcripts have been discovered also in A.thaliana, but enzymes involved in degradation of noncanonical caps have not been described so far. It has been shown that proteins belonging to NudiX family are involved in decapping of such transcripts in other organisms. A.thaliana genome includes as much as 28 genes of NudiX enzymes for which substrates are small molecules, such as varied nucleotides and their derivatives. Our studies were focused on plant AtNud6, AtNud7, AtNud19 and AtNud27 proteins and their capability of cleaving RNAs capped with various of caps e.g. NAD,

NADH, coenzyme A, ADP-ribose and NpnN. We demonstrated that most favoured substrate for AtNud6 and AtNud7 is ApaA-RNA, while AtNud19 is able to cleave NADH-RNA in vitro. We have also discovered that AtNud27 is the strongest and the most versatile decapping enzyme in vitro.

## 16

## Kinetic analysis of IFITs interactions and the role of IFITs complexes in IFIT1 binding to IVT mRNA

**Jingping Geng**, Magdalena Chrabąszczewska, Karol Kurpiejewski, Marzena Jankowska-Anyszka, Anna Stankiewicz-Drogon, Edward Darzynkiewicz, Renata Grzela

Interdisciplinary Laboratory of Molecular Biology and Biophysics, Centre of New Technologies, University of Warsaw, Poland

IVT mRNA has emerged as a promising drug candidate for various diseases, including cancer, genetic disorders and infectious diseases. For example, mRNA vaccines against COVID-19 represent a significant success in the field of RNA therapeutics. Despite this progress, foreign RNAs trigger a potent cellular antiviral response, resulting in robust upregulation of interferon-induced proteins with tetratricopeptide repeats (IFITs), which in turn leads to RNA translation restriction. IFIT1 plays a major role in recognizing and binding to 5' end of nonself-RNA. Murine IFIT family members of IFIT1, IFIT2, and IFIT3 are known to interact with each other, forming biological hetero-complexes, involved in the regulation of antivirus immune responses. However, the approximate binding affinity constants between these three IFITs and the role of IFITs complexes in the recognition and binding of IFIT1 to different synthesized capped RNA has not been elucidated. Here, we determined biophysical data on the interactions among IFITs and the binding kinetic parameters of IFIT1 to differently capped mRNAs in the presence of IFITs complexes. We analyzed thermal stability of single IFITs and IFITs complexes, demonstrating that IFITs complexes are more thermally stable than single IFITs. MST protein interaction assay proved that IFIT1 shows strong binding affinity to IFIT3 and the highest affinity is observed for IFIT1 to IFIT2/3 complex. Finally, results from BLI interaction assay revealed that complex formation among IFITs increase the binding affinity of IFIT1 to mRNA containing cap0 and cap1, providing valuable insights to the understanding of molecular mechanism of mRNA recognition by innate immune system.

## 17

## Potential interaction of PNRC1 protein with proteins forming the decapping complex

**Milena Krajewska**, Zuzanna Napora, Edward Darżynkiewicz, Renata Grzela

Centre of New Technologies, University of Warsaw, Poland

PNRC1 (a proline-rich nuclear receptor coregulatory protein 1) is described as a co-activator of nuclear receptor, a negative regulator of the RAS signaling pathway, but also as a novel regulator of rRNA maturation. It was shown that PNRC1 recruits the decapping complex to the nucleolus by interaction with the cytoplasmic, acting in specialized structures called processing bodies (P-bodies), DCP1α/DCP2. There, the DCP1α/DCP2 decapping machinery accumulates alongside RNA-degrading enzymes and their substrate RNAs. PNRC1 paralog - PNRC2 is a factor involved in the mRNA decay pathways, which resides in the cytoplasm where it interacts with DCP1α inside P-bodies. The proline-rich region of

PNRC2 is bound to the EVH1 domain of Dcp1a. PNRC1 r and PNRC2 show high sequence conservation. Both have a proline-rich Src homology domain 3 (SH3)-binding motif and a short hydrophobic motif (LXXLL-motif). We believe that PNRC1 may also interact with Dcp1 on this rule. In HEK293T cells using immunoprecipitation we tested whether the PNRC1 protein interacts with same proteins formed the decapping complex in homeostasis and after exposure to oxidative stress. In addition, we also supported our results using confocal microscopy, which allowed us not only to check the potential interaction, but also to determine the location of the proteins. We observed the interaction between PNRC1 and Dcp1. We also concluded that PNRC1 does not interact directly with Dcp2 and EDC4, as PNRC1 localizes in the cell nucleus, while EDC4 localizes in the cytoplasm.

This work was supported by Polish National Science Center, grant Miniatura2 2018/02/X/NZ1/03481.

**18**

## microRNA-mediated dysregulation of transcription in Huntington's disease

**Emilia Kozłowska**, **Agata Ciołak**, Grażyna Adamek, Julia Szcześniak, Agnieszka Fiszer

Department of Medical Biotechnology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by expansion of CAG repeats in exon 1 of the huntingtin gene (HTT), resulting in abnormally long polyglutamine (polyQ) tract in encoded protein. Transcriptional dysregulation is an early event in the course of disease and has important implications to HD pathogenesis. In this study, we aimed to describe specific molecular pathways leading to transcriptional alterations in HD, including disruptions resulting from both, mutant HTT expression, as well as haploinsufficiency of normal HTT.

We employed RNA-Seq (including small RNAs) to analyze set of isogenic, iPSCs-derived neural stem cells (NSCs). We observed significant changes in genes expression, as well as substantial dysregulation of miRNAs, in HD cell line and in HTT knockout, as compared to control line. GO enrichment analyses of differentially expressed genes revealed that up-regulated genes in HD cells were mostly associated with DNA binding and regulation of transcription. We reported significant up-regulation of expression of transcription factors (TFs) and transcription regulators: TWIST1, SIX1, TBX1, TBX15, MSX2, MEOX2, DBX1 and FOXD1 in NSCs and medium spiny neurons-like cells. Moreover, we identified miRNAs: miR-214, -199a, and -9 as co-regulators of the level of specific transcripts together with TFs by feed-forward regulatory loop. Based on these results we propose new molecular pathway implicated in HD pathogenesis.

This work was supported by the National Science Centre: [2015/17/N/NZ2/01916; 2015/17/D/NZ5/03443]

**19**

## NOL12 is required for pre-rRNA processing and regulates this process in response to stress in Arabidopsis

**Anna Golisz-Mocydlarz**, Monika Zakrzewska-Płaczek, Michał Krzysztoń, Justyna Piotrowska, Joanna Kufel

Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Poland

NOL12 5'-3' exoribonucleases, conserved among eukaryotes, play important roles in pre-rRNA processing, ribosome assembly and export. The best described yeast counterpart, Rrp17, is required for the maturation of 5.8S and 25S rRNAs, whereas human hNOL12 is crucial for the separation of the large (LSU) and small (SSU) ribosome subunit rRNA precursors. Here we show that plant AtNOL12 is also involved in rRNA biogenesis, particularly in the processing of the LSU rRNA precursor, 27S pre-rRNA. Importantly, the absence of AtNOL12 alters the expression of many ribosomal protein and ribosome biogenesis genes, which may further exacerbate rRNA biogenesis defects, or, alternatively, maybe the effect of the disturbed ribosome assembly caused by delayed pre-rRNA processing. Also, exposure of nol12 mutants to stress factors, including heat, salt and pathogen Pseudomonas syringae, enhances the observed molecular phenotypes, linking pre-rRNA processing to stress response pathways. AtNOL12-dependent aberrant rRNA processing may affect ribosome function, as indicated by improved mutant resistance to ribosome-targeting antibiotics.

**20**

## A new approach to studying Candida albicans mitochondrial transcriptome via direct RNA sequencing

**Jakub Piątkowski** and Paweł Golik

University of Warsaw, Poland

Nanopore sequencing presents the possibility of accessing a new, previously unavailable layer of information in RNA-seq data. While Nanopore platform has been used in whole-transcriptome direct RNA sequencing to great success, there are still relatively few projects employing this method specifically for studying organellar transcriptomes. Organellar transcriptome sequencing also comes with its own methodological challenges, both in the experimental and data analysis stage. This work comprises Nanopore sequencing of Candida albicans mitochondrial transcriptome along with development of a dedicated data analysis pipeline, allowing for discovery and quantification of complete transcript isoforms and posttranslational RNA modifications, as well as a preliminary analysis of C. albicans deletion mutants in genes encoding for 5' and 3' mitochondrial RNA exonucleases CaPET127 and CaDSS1. Full-length direct RNA sequencing on Nanopore platform detects both quantitative and qualitative changes in mitochondrial RNA splicing and modifications between the mutant strains, as well as each mutant strain and the wild-type strain.

## 21

## TENT5-mediated cytoplasmic polyadenylation of mRNAs encoding secreted proteins is essential for oogenesis in mice

**Michał Brouze**, Monika Kusio-Kobiałka, Olga Gewartowska, Agnieszka Czarnocka-Cieciura, Marcin Szpila, Seweryn Mroczek, Bartosz Tarkowski, Kamil Jachacy, Andrzej Dziembowski

International Institute of Molecular and Cell Biology in Warsaw, Poland

Cytoplasmic polyadenylation is established as one of the most important mechanisms controlling and regulating oogenesis. However, while the best-described cytoplasmic poly(A) polymerase TENT2 (GLD2) was shown to be essential for oogenesis and development in invertebrates, its knock-out mutation doesn't produce any phenotype in mice. Here we demonstrate that TENT5 family of poly(A) polymerases can provide an alternative polyadenylation pathway.

TENT5B and TENT5C are highly expressed in germinal vesicle stage oocytes. Knock-out mutation of both genes (but not individual ones) leads to female infertility through oocyte development arrest followed by degeneration of all oocytes in adult females. Additionally, in contrast to single Tent5b knock-out, which does not affect fertility, C-terminal tagging of TENT5B with GFP leads to strong infertility in females, manifested by an extreme drop in litter sizes caused by chromosomal segregation errors in ovulating oocytes, strongly suggesting a gain of function effect.

Poly(A) tail length profiling using Direct RNA sequencing on MinION platform revealed that TENT5B and TENT5C polyadenylate mRNAs encoding proteins targeted to the endoplasmic reticulum. Notably, we discovered the opposite effect of Tent5b/c double knock-out and Tent5b GFP knock-in, as the same groups of transcripts had shortened poly(A) tails in the first case but elongated in the latter. The shortening of poly(A) tail also resulted in a radical drop of the expression of the encoded proteins. Identified substrates don't share any common sequence motives. Notably, ER-leader sequence proved to be a determining factor for polyadenylation by TENT5B and TENT5C, as evidenced by mRNA reporter assay in oocytes.

## 22

## hBM-MSCs engineering with ARCA-capped mRNA

**Anna Stankiewicz-Drogon**, Renata Grzela, Siranjeevi Nagaraj, Anna Andrzejewska, Piotr Rogujski, Edward Darzynkiewicz, Barbara Łukomska, Mirosław Janowski

University of Warsaw, Poland

Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) are widely used for treatment in experimental degenerative diseases. Since the use of naïve MSCs as an effective approach has not been proven, different methods were applied to modify MSCs before transplantation. mRNA seems to meet the needs of MSCs modification for clinical applications perfectly. 5' cap modification with antireverse cap analog (ARCA) and its derivatives ensures stability and high levels of mRNA translation in the cell.

hBM-MSCs were transfected with in vitro transcribed (IVT) pseudouridinylated mRNA (ΨU-mRNA) encoding Firefly luciferase gene and capped with anti-reverse cap analogs: ARCA 3', β-S-ARCA D1 and

β-S-ARCA D2. To assess the translational efficiency of the reporter gene, a bioluminescent assay was used, while the MSCs' metabolic activity was measured in the CCK-8 assay. Finally, the expression of genes encoding proteins involved in innate and cellular immune response was evaluated by qRT-PCR.

In our studies, luciferase activity in hBM-MSCs was markedly increased after transfection with HPLC-purified ΨU-mRNAs. qRT-PCR analysis revealed strong upregulation of IFIT1, OAS1, MX1, RIG-I, MDA5, IFIT5, and NOD2 genes exclusively in cells trasfected with crude ARCA 3'-capped mRNA and only in this case a significant change in metabolic activity was observed. Overall, we demonstrated that the transfection with ARCA-capped HPLC-purified ΨU-mRNAs is an efficient and safe strategy for hBM-MSCs engineering, ensuring increased stability and translational efficiency of the transcript as well as minimizing immunogenicity in the cells.

---

**23**

## A long non-coding antisense transcript of DOG1 regulates Arabidopsis thaliana secondary seed dormancy by BRM, a SWI/SNF ATPase

**Magdalena Wrona**, Julia Zinsmeister, Ruslan Yatusevich, Szymon Świeżewski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The DOG1 (Delay of Germination 1) gene is a key regulator of seed dormancy and germination in plants. Its expression is controlled by various molecular mechanisms, including a cis-acting antisense transcript (asDOG1) that acts as a negative regulator of dormancy. We investigated the role of the SWI/SNF chromatin remodeling complex in modulating the expression of DOG1 and asDOG1 during secondary dormancy induction by darkness and high temperature. We found that the BRM ATPase subunit of the SWI/SNF complex negatively regulates seed dormancy by affecting the levels of both DOG1 and asDOG1 transcripts. Our ChIP, FAIR, RT-qPCR and phenotypic results suggest that BRM ATPase regulates DOG1 gene expression through antisense transcription. We also show that BRM ATPase specifically affects secondary dormancy, which is induced by unfavorable environmental conditions after seed imbibition, but not primary dormancy, which is established during seed maturation.

---

**24**

## FinO-domain protein ProQ from Neisseria meningitidis recognizes intrinsic transcription terminators in its RNA targets

**Maciej M. Basczok** and Mikołaj Olejniczak

Institute of Molecular Biology and Biotechnology, Department of Gene Expression, Adam Mickiewicz University, Poznań, Poland

The FinO-domain proteins belong to the RNA-binding proteins in bacteria. Although FinO-like proteins vary greatly in size and composition, all members possess a structurally similar FinO domain, which constitutes their main RNA-interacting portion. So far several members of this protein family have been extensively studied including Neisseria meningitidis minimal ProQ. Although the protein was demonstrated to interact in vivo with many transcripts, similarly to other FinO-domain proteins it lacks apparent sequence specificity. Instead, it displays a strong preference for 3'-UTR regions, suggesting

that intrinsic terminator structures might be preferred. It raises an interesting question about the details of these interactions.

Our in vitro analysis showed that N. meningitidis ProQ specifically recognizes and binds transcripts of different origin (3'-UTRs, 5'-UTR, sRNAs) and the interaction is primarily mediated via intrinsic terminator-like structures, even if located in 5'-UTRs. Shortening of the 3'-oligo(U) tail below six uridines weakens the rpmG-3'UTR and AniS RNAs affinity to Nm ProQ. On the other hand, the extension of the oligo(U) tail much above six uridines of length weakens these interactions. The RNA sequence on the 5' side of the terminator hairpin of at least six nucleotides is needed for efficient rpmG-3'UTR binding to Nm ProQ. Finally, shortening the terminator stem of rpmG-3'UTR showed a negative impact on the binding strength. Overall, our studies demonstrate that the efficient binding of RNAs to N. meningitidis ProQ is mediated not only by the intrinsic terminator stem but also by oligo(U) tail and sequence on the other side of the terminator hairpin

**25**

## Arabidopsis homolog of human CWC22 is required for seed development

**Martyna Koźbiał**[1], Helena Kossowska[1], Marta Gapińska[1], Roksana Iwanicka-Nowicka[1,2], Wojciech Wróblewski[1], Marta Góra[1], Michał Krzysztoń[2], Szymon Świeżewski[2], Małgorzata Lichocka[2], Antoni Palusiński[1], Katarzyna Sosnowska[1], Marta Koblowska[1,2]

[1] Laboratory of Systems Biology, Faculty of Biology, University of Warsaw, Poland

[2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Splicing of precursor mRNA (pre-mRNA) is a key step in gene expression in eukaryotes, increasing the diversity of mRNAs expressed from the genome, enriching the diversity of protein products and their functions. CWC22 is a well-known general splicing factor in human cells, essential for pre-RNA splicing. Here, we show that the Arabidopsis homolog of human CWC22 encodes a protein with strong evolutionary conservation within the two domains MIF4G and MA3 and conservation of protein structure based on comparison of available experimental structures and Alphafold models. Analysis of an Arabidopsis T-DNA mutant in the gene encoding AtCWC22 revealed that the protein is essential for plant survival, as no homozygotes were found in the pool of developed seedlings. It was observed that about 25% of the seeds collected from a heterozygous plant did not germinate. Macroscopic analyses of mature siliques showed a similar percentage of defective seeds. Nomarski interference contrast led to the precise determination of a developmental defect during embryogenesis at the late globular stage of the embryo in cwc22 -/- seeds. At the same time, an analogous analysis of insertion mutants in two other genes encoding proteins involved in RNA splicing - an unexplored homolog of the human AtCWC21 protein and the cyclophilin CYP95 with probable functions in plant splicing - was performed. In order to find genes regulated by AtCWC22, transcriptome analysis was performed using the single-seed RNA-seq method. Seeds of cwc22 +/-, cwc21 -/- and cyp95 -/- were used for the experiment.

**26**

## Splicing factor dynamics in steady-state and malignant hematopoiesis

Vladyslava Liudkovska, Kevork Wakimian, Ashwini Khaladkar, Phuong Cao Thi Ngoc, Cristian Bellodi, **Maciej Cieśla**

IMol, Polish Academy of Sciences, Warsaw, Poland

Splicing is a rapid amplifier of coding capacity of the genome. This fundamental biological process is governed by a multi-modular, ribonucleoprotein machinery of spliceosome. Large-scale, genome-wide studies indicate malfunctions in spliceosome as drivers of abnormal differentiation of blood, during process of hematopoiesis. However, underlying molecular pathways remain poorly defined.

In this study we chart the splicing factor (SFs) dynamics during normal and malignant hematopoiesis. We define cohorts of SFs regulated at post-transcriptional level. Furthermore, we identify core SF, SF3B1 as subjected to regulatory changes during leukemic transformation. This is achieved through a conserved epitranscriptomic program that steers SF3B1 levels to counteract leukemogenesis. Our analysis of human and murine pre-leukemic hematopoietic cells reveals dynamic regulation of SF3B1 protein abundance, which impacts myelodysplastic syndrome-to-leukemia progression in vivo. Mechanistically, ALKBH5-driven 5'UTR m6A demethylation fine-tunes SF3B1 translation directing splicing of central DNA repair and epigenetic regulators during transformation. This impacts genome stability and leukemia progression in vivo, supporting integrative analysis in humans that SF3B1 molecular signatures may predict mutational variability and poor prognosis. Collectively, these findings highlight a post-transcriptional gene expression nexus that unveils unanticipated SF3B1-dependent cancer vulnerabilities in blood differentiation.

---

**27**

## HuR silencing promotes Retinal Ganglion Cells degeneration and alleviates the activity of exogenous neuroprotection in glaucoma

**Anna Pacwa**, Joanna Machowicz, Piotr Rodak, Bartosz Machna, Saeed Akhtar, Marialaura Amadio, Joanna Lewin-Kowalik, Adrian Smędowski

Medical University of Silesia in Katowice, Poland

Purpose: To evaluate the impact of HuR gene silencing on the ratio of age-related degeneration of Retinal Ganglion Cells (RGC), which potentially describes the efficiency of endogenous neuroprotection mechanisms, as well as to assess the exogenous neuroprotection capacity of HuR-silenced RGC in rat glaucoma model treated with metallothionein.

Methods: Thirty-five eight-week-old Long Evans rats were divided into two groups: experimental and control. Experimental group received intravitreal injection of AAV-shRNA-HuR. Control group received AAV-shRNA-control. Animals were sacrificed in 3 different time points. Healthy and treated retinas were collected and processed for immunostainings and RGC count. During the experiment electroretinography tests (ERG) were performed. For the second trial, 8 weeks after AAV injection, unilateral episcleral vein cauterization was performed to induce glaucoma model. Half of animals

received metallothionein (MT). During the experiment IOP was monitored and ERG tests were performed. Retinas were collected for immunostainings and RGC count.

Results: RGC count was 310±31, 296±25, 189±41 in experimental group and 399±51, 395±49, 390±23 in control respectively for 2, 4 and 6 months after injection. (Kaplan-Mayer trend rank $p<0.0001$). Loss of RGC in central retina was 33.7% in animals from shRNA-HuR MT-treated glaucoma and 11.4% in shRNA-control, MT-treated glaucoma ($p<0.05$). In peripheral part of the retina the loss was 37.4% in animals from shRNA-MT-treated glaucoma and 11.5% in shRNA-control-MT-treated glaucoma ($p<0.01$).

Conclusion: Silecing of HuR gene enhanced age-related loss of RGC which translates into impaired endogenous neuroprotection pathways morover the absence of HuR critically limits neuroprotective activity of exogenously delivered metallothionein.

---

**28**

## Genome-wide analysis of chromatin signature/composition at human transcription termination regions

**Magda Kopczyńska**, Chihiro Nakayama, Agata Stępień, Takayuki Nojima, Kinga Kamieniarz-Gdula

Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznań, Poland

Transcription of protein coding genes by RNA polymerase II (Pol II) can be affected by multiple chromatin features. However, our knowledge of how chromatin remodelers and histone modifications affect the last step of this process, transcription termination, is very limited. Thus, we asked two questions: 1) what is the chromatin signature of human terminators and 2) how do transcription termination and chromatin shape each other?

We used T4ph mNET-seq datasets obtained from HeLa cells as an input to comprehensively define termination regions genome-wide. Using publicly available datasets for this cell line, we performed in silico data mining for a wide range of chromatin-associated features including histone modifications, transcription factors, and chromatin remodelers. We found that many factors are enriched in termination regions, and the pattern of binding differs depending on their localization. Moreover, we are currently investigating the mechanism underlying the role of H3K36me3 in termination. The detailed results of the analyses will be presented.

In summary, we computationally studied the chromatin environment, forming termination regions in an unbiased manner. In silico data mining uncovered transcription factors associated with termination that could potentially form a roadblock for transcribing Pol II, as described previously in yeast.

---

29

## Yeast-based factory for therapeutic mRNA bioproduction

**Małgorzata Zawadzka**, Leszek Błaszczyk, Urh Černigoj, Fayza Daboussi, Mara G. Freire, Julita Gumna, Katarzyna Pachulska-Wieczorek, Mario Perkovic, Rok Sekirnik, Fani Sousa, Chantal Pichon

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

The field of mRNA therapeutics is rapidly expanding, with numerous applications being developed and tested. The most recognized example is the widespread usage of mRNA vaccines to combat the COVID-19 pandemic. However, to date, the production of mRNA therapeutics is mainly based on in vitro methods, which are limited by high manufacturing costs and difficult production of large-size mRNA.

The Yscript consortium aims to develop a cost-effective, large-scale mRNA production platform for the efficient production of mRNA therapeutics and vaccines. To produce mRNA, we plan to use yeast, that are well-established in industrial biotechnology. Our strategy involves isolating the mRNA of interest by packing it into specific foci within yeast cells, followed by its extraction and purification. The efficacy and safety of the yeast-produced mRNA therapeutics will be assessed through rigorous quality control analysis of structure and efficacy.

Overall, the Yscript consortium's research has the potential to revolutionize the production of mRNA therapeutics and vaccines, making them more affordable and easier to manufacture and thus accessible to a wider population in a fast, safe, and reliable way. Moreover, the development of a yeast-based mRNA bioproduction platform could significantly impact the ongoing global response to emerging viral threats.

Yscript has received funding from the European Innovation Council (EIC) under grant agreement No 101047214.

---

30

## Nonconventional introns as factors affecting the coding potential of euglenid genes

**Maria Jagielska**, Paweł Hałakuc, Rafał Milanowski

Institute of Evolutionary Biology, Faculty of Biology, University of Warsaw, Poland

Euglenids genomes remain poorly understood, due to their large sizes, complexity and repetitiveness. So far only a draft genome of Euglena gracilis has been published. One of the remarkable features of euglenids genomes is the presence of distinct type of introns, so called nonconventional introns. This type introns differ from spliceosomal ones, common in nuclear genes of eukaryotes including euglenids. Ranging from tens of bases to several kilobases, they have non-canonical borders, lack polypyrimidine tract and form a stable, yet weekly preserved RNA secondary structure bringing together intron ends, and the ends of adjacent exons. Moreover, these atypical introns can be inserted at new positions, and are rapidly removed from transcripts (in circular form) but later than conventional ones, most likely post-transcriptionally, by unknown mechanism of removal.

Analyses of genomic and transcriptomic sequences from three species (E. gracilis, E. hiemalis, E. longa) have shown other peculiarities related to nonconventional introns, which were subsequently tested and confirmed experimentally: for example, the occurrence of additional introns within existing ones (twintrons). It has also been shown that nonconventional introns can be alternatively excised. This phenomenon relies on recognizing alternative borders of introns in pre-mRNA, which result in different mRNA, coding for different proteins, making the nonconventional introns evolutionary hotspots favoring creation of new proteins. Such discovery shows the influence of these introns on repertoire of proteins in contemporary euglenids, but could also suggest a similar role of spliceosomal introns in early eukaryotes, before the spliceosome evolved into very precise machinery.

**31**

## Detection and quantification of RNA modifications using UPLC-MS/MS

**Marta Starczak**, Fabian Leśniewski, Maciej Gawroński, Daniel Gackowski

Nicolaus Copernicus University in Toruń, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Department of Clinical Biochemistry, Faculty of Pharmacy, Bydgoszcz, Poland

RNA harbors the potential of being dynamically and reversibly regulated by adding and removing distinct chemical moieties, which extends the RNA repertoire and alters its chemistry in various ways. To date, over one hundred post-translational RNA modifications have been identified. R-loops are three-stranded nucleic acid structures consisting of an RNA/DNA hybrid and an unpaired strand of non-template DNA that represent a significant source of genomic instability and are involved in regulating several critical biological processes in eukaryotic cells. A growing body of experimental evidence suggests that RNA moieties of RNA/DNA hybrids may convey RNA modifications influencing various aspects of R-loop biology.

Here we present a method for analysis of the broad spectrum of RNA modifications using stable-isotope dilution ultra-performance liquid chromatography coupled with tandem mass spectrometry. Such an approach provides the most unambiguous method to detect and quantify modified nucleotides. Our method enables the quantitative analysis of 5-methylcytidine, 5-hydroxymethylcytidine, 5-methyluridine, 5-hydroxymethyluridine, 5-formyluridine, and N6-methyladenosine in RNA from yeasts, plants, animals and in synthetic oligonucleotides.

Moreover, the protocol involving RNAase H treatment makes distinguishing modifications from RNA and RNA/DNA hybrids possible. After digestion with RNase H, we were able to determine the levels of N6-methyladenine and 5-methylcytidine in material delivered from cell lines, as well as in PBMC from leukemia patients. However, we were unable to determine the 5-hydroxymethylcytidine level as it was below the detection limit of our method.

**32**

## Identification of cellular factors associated with the bunyaviral transcription and translation

**Moh Egy Rahman Firdaus**, Eliana Dukhno, Piotr Gerlach

Laboratory of Structural Virology, IMol, Polish Academy of Sciences, Warsaw, Poland

Bunyaviruses are neglected pathogenic RNA viruses, posing a persistent threat to global health. The current lack of preventive and therapeutic options against bunyaviruses makes drug and vaccine development a pressing issue. In our research, we use Bunyamwera (BUNV) and La crosse (LACV) model orthobunyaviruses to study the host translation shut-off and its reprogramming during bunyaviral infection. Moreover, we aim at identification of the host factors essential for the bunyaviral translation, hoping that they could be used to design host-based, broad-spectrum antivirals. We use reporter-based mini-replicon system, supported by the use of viral-like particles, to mimic viral transcription and translation in human cell lines. We carefully engineered affinity tags and/or TurboID ligase into bunyaviral proteins L, N, and NSs, to identify their direct and indirect host interacting partners. We extensively optimized the system using multiple promoters, and checked whether tagged and fusion versions of the viral proteins do not interfere with viral transcription and translation. In addition, we utilize the N-terminal dsRNA-binding domain of the PKR kinase, competing with the native PKR for the viral RNA, thus attenuating the anti-viral response. Knowledge gained from this research will not only broaden our understanding of the bunyaviral infection on the molecular level, but it will also set the basis for the design of new therapies and broad-spectrum antiviral drugs.

---

**33**

## Selection of miRNAs specific to exosomal membranes

**Dominika Maziarz**, Karolina Sapoń, Teresa Janas, Tadeusz Janas

Institute of Biology, University of Opole, Poland

Studies on the interaction of small RNA molecules with membranes suggest that RAFT and EXO motifs are important elements of this binding but unfortunately, the exact molecular mechanism is still unknown. The research conducted so far has included research on the selective binding of RNA to the MVB membrane, which can be regulated by activity of the lipid raft region in the MVB membrane and the presence of RNA motives.

The aim of this research is to test the hypothesis that the RAFT and EXO motifs are important for the binding of miRNA molecules to membranes.

Firstly, the selection of miRNAs that can bind to exosomal liposomes is performed. The technique of gel filtration is used for this purpose. The pool of 13 different miRNAs is chosen based on the presence of different motifs within their sequences. After making the selection, the obtained RNAs (co-eluted with liposomes) are isolated and their concentration is measured using the real-time PCR technique.

Next miRNAs are tested for their interaction with model membranes (exosomal liposomes) and exosomes by using measurements of dissociation constant (KD) values of RNA-liposome complexes and the FRET technique. The obtained results are analyzed in terms of their correlation with the motifs found in the miRNAs.

**34**

## New insights into the complexity of SCA3: the contribution of non-coding RNAs

**Magdalena Jazurek-Ciesiołka**, Adam Ciesiołka, Emilia Kozłowska, Grzegorz Figura, Michał W. Szcześniak, Edyta Kościańska, Paweł Joachimiak, Grażyna Adamek, Izabela Makałowska, Agnieszka Fiszer

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the ATXN3 gene and subsequent polyglutamine tract in ataxin-3 protein. Although the underlying mutation has been known for almost 30 years, the complexity of its effects is still under extensive exploration. Recent studies suggest that non-coding RNAs (ncRNAs) might be implicated in the pathogenesis of neurodegenerative disorders, including repeat expansion diseases. In the present study, to gain insight into the toxicity induced by mutant ataxin-3, we examined the potential contribution of long ncRNAs (lncRNAs) and microRNAs (miRNAs) to SCA3. We developed a novel isogenic models in SH-SY5Y cells (using Flp-In T-REx system), stably expressing full-length ATXN3 with expanded CAG repeats (120CAG, SCA3 model) or with nonpathogenic CAG repeats (20CAG, control). As a result of lncRNAs and miRNAs profiling through RNA-Seq analysis, a total of 633 differentially expressed lncRNAs (200 upregulated and 433 downregulated) and 94 differentially expressed miRNAs (44 upregulated and 50 downregulated) were identified in SCA3 model. GO analysis showed that DE lncRNAs were enriched in a variety of biological processes including: Translation, Ribonucleoprotein complex biogenesis, Nucleocytoplasmic transport or Autophagy. Whereas KEGG pathway analysis revealed that DE miRNAs were involved in processes such as Axon guidance, GABAergic synapse, Hippo signaling, Wnt signaling and Ubiquitin mediated proteolysis. Based on bioinformatic analyzes and validation results, promising candidates involved in SCA3 pathogenesis were selected among lncRNAs: AC051619.7, BCYRN1, H19, TERC, SMARCA5-AS1, ZFPM2-AS1 and miRNAs: hsa-miR-490-3p, hsa-miR-218-5p, hsa-miR-222-3p, hsa-miR-296-5p, hsa-miR-221-3p, hsa-miR-34c-5p, hsa-miR-34b-5p.

35

## Effect of polysialic acid and galacturonic acid on RNA binding to exosomal membrane

**Iga Gawrońska**, Karolina Sapoń, Teresa Janas, Tadeusz Janas

Institute of Biology, University of Opole, Poland

Exosomes have been extensively explored as delivery vehicles due to high efficiency and specificity in delivering their cargo which includes proteins, lipids, and nucleic acids from the donor cells. Exosome mimicking liposomes (exosomal liposomes) were formulated based on the lipid concentration in exosomal membrane.

Polysialic acid (polySia) naturally occurs on the surface of exosomes originating from cancer cells. PolySia is an anionic polysaccharide and is known to be related to certain diseases (mental, neurodegenerative disorders and cancer). In our experiments we are using also another anionic polysaccharide (galacturonic acid) to show its impact on binding an RNA aptamers to biological membranes and compare to the effect of polySia.

The mechanism of binding RNA molecules to membranes is still unknown. The RNA aptamer that is presented contains EXO-motif and RAFT-motifs which can also promote interactions with exosomes. EXO-motif is most frequently found in exosomal RNA, RAFT-motifs are most frequently found in RNA aptamers specific for lipid rafts. Location of RNA motifs can be predicted by using the m-fold program and analyzing the secondary structure of RNA sequence. Exosomal membranes and RNA molecules are labelled using membrane specific and RNA specific fluorescence probes. Based on FRET measurements the dissociation constant (KD) of RNA-membrane complex is calculated showing the RNA aptamer affinity to exosomal membrane and to exosomal liposome membrane.

# Poster Session II

| Poster No | Authors | Title |
|---|---|---|
| 36 | **Klaudia Staszak**, Magdalena Kubiak, Michał Szcześniak, Izabela Makałowska | Non-coding retrogenes in human cancers |
| 37 | **Alicja Armatowska**, Eliza Molestak, Marek Tchórzewski, Stefan Bresson, Jan Słupski, Magdalena Boguta, Małgorzata Cieśla | The linkage between Maf1-mediated control of tRNA transcription and mRNA translation in yeast Saccharomyces cerevisiae |
| 38 | **Aleksandra Łopusińska**, Michał Tys, Magdalena Boguta, Małgorzata Cieśla | Regulation of the RNA polymerase III transcription machinery is dependent on the ubiquitin ligase Rsp5 |
| 39 | **Agnieszka Piasecka**, Michał Sekrecki, Arkadiusz Kajdasz, Michał W. Szcześniak, Krzysztof Sobczak | Microtranscriptome of skeletal muscles is regulated by MBNL splicing factors |
| 40 | **Ewa Tykwińska**, Angelika Andrzejewska-Romanowska, Paweł Śledziński, Julita Gumna, Katarzyna Pachulska-Wieczorek | Transcriptome-wide analysis of yeast RNA secondary structures |
| 41 | **Malak Farhat**, Aleksandra Łopusińska, Ewa Leśniewska, Małgorzata Cieśla, Magdalena Boguta | Functional suppression of maf1 deletion mutant of Saccharomyces cerevisiae by overdose of N-terminal domains of the largest Pol III subunit, C160 |
| 42 | **Natalia Gumińska**, Katarzyna Matylla-Kulińska, Wiktoria Orzeł, Paweł Krawczyk, Michał Maj, Seweryn Mroczek, Andrzej Dziembowski | Detection of non-adenosine residues within poly(A) tails using Oxford Nanopore direct RNA sequencing & deep learning |
| 43 | **Angelika Andrzejewska-Romanowska**, Julita Gumna, Ewa Tykwińska, Katarzyna Pachulska-Wieczorek | Discovering structural features of the active Ty3 retrotransposon RNA genome |
| 44 | **Karolina Kasztelan**, Aleksander Chlebowski, Tomasz M. Kuliński, Kamila Affek, Andrzej Dziembowski, Roman J. Szczęsny | Identification of proteins involved in the regulation of double-stranded RNA level in the nucleus of human cell |
| 45 | **Joanna Zwolenkiewicz** and Mikołaj Olejniczak | The role of the ProQ protein in RNA-RNA interactions involving small RNAs in Escherichia coli |
| 46 | **Przemysław Latoch**, Olga Iwańska, Mariia Kovalenko, Agata Starosta | TimeSeqR: a comprehensive R package for integrated analysis of transcriptomic and translatomic data from time-course RNAseq and RIBOseq experiments |
| 47 | **Alexander Hammermeister**, Agnieszka Broniec, Sebastian Glatt | Structure of the human tRNA methyltransferase complex FTSJ1-THADA |
| 48 | **Patrycja Świergiel** | Elucidating the molecular mechanisms underlying the synthesis of sdRNAs mediated by FUS |
| 49 | **Eliana Dukhno**, Moh Egy Rahman Firdaus, Piotr Gerlach | Impact of the bunyaviral mRNA 3'UTR on transcription and translation |

| | | |
|---|---|---|
| **50** | **Jan Mikołajczyk** and Tomasz W. Turowski | Mapping Active Transcription Sites of Human RNA Polymerase III at Nucleotide Resolution |
| **51** | **Anna Barczak-Brzyżek**, Grzegorz Brzyżek, Marek Koter, Ewa Siedlecka, Piotr Gawroński, Marcin Filipecki | Chloroplast retrograde control of miRNA expression in response to high light stress |
| **52** | **Paweł Konieczny**, Mirosław Cebula, Marta Kot, Klaudia Skrzypek, Marcin Majka | miR-30a conjugated with cholesterol as a novel therapeutic approach in the treatment of rhabdomyosarcoma |
| **53** | **Bart Krist**, Tansu Doran, Katarzyna Czerniak, Yaarob Altalli, Michal Gdula | Epigenetic control of transcription during keratinocyte differentiation |
| **54** | **Gabriela Machaj**, Dariusz Grzebelus, Guillem Ylla | The transcriptomic basis of rhizomania resistance in sugar beet |
| **55** | **Nikola Musiała** and Ewa Stępniak-Konieczna | Endogenous tuning of MBNL1 corrects alternative splicing defects in a cell model of myotonic dystrophy type 1 (DM1) |
| **56** | **Anna Piaszyk-Borychowska**, Kamil Wójcik, Katarzyna Czerniak, Michał Gdula | Role of SmcHD1 in the transcription inactivation and replication timing shift on female X chromosome |
| **57** | **Marta Walczak** and Sebastian Glatt | Structural and functional characterization of the human tRNA thiolation cascade |
| **58** | **Kinga Gołębiewska** and Piotr Gawroński | Arabidopsis Era1 GTPase homolog is involved in 16S rRNA maturation in chloroplasts |
| **59** | **Ewelina Kałużna**, Julian Zacharjasz, Monika Piwecka | Spatial transcriptomics of mouse pituitary gland |
| **60** | Renata Grzela, **Karolina Piecyk**, Anna Stankiewicz-Drogon, Paulina Pietrow, Maciej Lukaszewicz, Karol Kurpiejewski, Paulina Skrzypczyk, Eliza Rajkowska, Edward Darzynkiewicz, Marzena Jankowska-Anyszka | Superior properties of mRNA capped with N2 modified dinucleotide cap analogs |
| **61** | **Kevork Wakimian**, Vladyslava Liudkovska, Maciej Cieśla | Function of SF3 spliceosome toward function of skeletal muscle progenitors |
| **62** | Akanksha Jaiswar, Dawid Walerych, **Maria Grześ** | A comprehensive transcriptome analysis reveals a novel catalog of long non-coding RNAs dependent on driver oncogenes (CMYC, KRAS, mutant p53) |
| **63** | **Maria M. Guzewska**, Krzysztof J. Witek, Elżbieta Karnas, Michał Rawski, Ewa Zuba-Surma, Monika M. Kaczmarek | miR-125b-5p – a mediator of EV biogenesis and secretion at the embryo-maternal interface |
| **64** | Pietro Boccaletto, Filip Stefaniak, Angana Ray, Andrea Cappannini, Sunandan Mukherjee, Elżbieta Purta, Małgorzata Kurkowska, Niloofar Shirvanizadeh, Eliana Destefanis, Paula Groza, Gülben Avşar, Antonia Romitelli, Pınar Pir, Erik Dassi, Silvestro G. Conticello, Francesca Aguilo, **Janusz M. Bujnicki** | MODOMICS: a database of RNA modification pathways |

| | | |
|---|---|---|
| **65** | **Sunandan Mukherjee**, Masoud A. Farsani, Matthias Bochtler, Janusz M. Bujnicki | SimRNA-Cry: a tool for modeling RNA molecules using maps from cryo-electron microscopy |
| **66** | **Andrea Cappannini**, Kevin Mosca2, Sunandan Mukherjee, S. Naeim Moafinejad, Richard R. Sinden, Veronique Arluison, Janusz M. Bujnicki, Frank Wien | NACDDB: Nucleic Acid Circular Dichroism Database |
| **67** | **Angana Ray**, Niloofar Shirvanizadeh, Iswarya Pandara Nayaka PJ, Sunandan Mukherjee, Masoud Amiri Farsani, Satyabrata Maiti, Janusz M. Bujnicki | A curated dataset of RNA and RNP 3D structures with biologically relevant assemblies, with chemically modified residues and their unmodified counterparts |
| **68** | Tales Rocha de Moura, Nagendar Goud Badepally, **Elżbieta Purta**, Agata Bernat, Katarzyna Merdas, Janusz M. Bujnicki | 3D structure determination of key regulatory RNAs at the 5' and 3’ untranslated regions of Flaviviruses |
| **69** | Paula Martin-Arroyo, Katarzyna Piętka, Natalia Stróżyńska, Grażyna Leszczyńska, Robert Nowak, **Małgorzata Adamczyk** | Development of methodology for quantitative analysis of tRNA modifications by nanopore RNA sequencing |
| **70** | **Sivakumar Krishnamoorthy**, Nathalie Idlin, Natalia Stec, Christos Spanos, Nila Roy Choudhury, Chen Zhuo, Juri Rappsilber, Gracjan Michlewski | HuR-targeted Eltrombopag and its implications on autophagy regulation |

**36**

## Non-coding retrogenes in human cancers

**Klaudia Staszak**, Magdalena Kubiak, Michał Szcześniak, Izabela Makałowska

Adam Mickiewicz University, Poznań, Poland

The retroposition process, in which a mature mRNA undergoes reverse transcription and a resulting cDNA is integrated back into the genome, leads to the formation of a new intronless gene copy known as a retrocopy or a retrogene. This process was especially potent during the evolution of primates and the retrocopies themselves have been named the "seeds of evolution". Despite the initial comprehension that they are pseudogenes, numerous of them are functional. Interestingly, only a small fraction of retrocopies encodes protein, while the vast majority is transcribed and potentially acts as non-coding RNAs. They play multifaceted roles within tumor cells, as they have been shown to be both oncogenes and tumor suppressors.

The aim of the study was to determine the repertoire of retrogenes that are differentially expressed in cancer samples and to indicate time of these retrocopies' origin during animal evolution. The RNA-Seq data from human were obtained from publicly available resources and further analyzed. In total we have identified around 40 retrocopies whose expression levels significantly change in tumors. The expression of selected retrogenes was confirmed in human cell lines and in human tissue cDNA panel. We have found both evolutionarily old retrocopies common to genomes of various animal species and group of young, potentially human-specific non-coding retrogenes. The presented study may be essential for better understanding the high rate of neoplastic processes in human.

**37**

## The linkage between Maf1-mediated control of tRNA transcription and mRNA translation in yeast Saccharomyces cerevisiae

**Alicja Armatowska**, Eliza Molestak, Marek Tchórzewski, Stefan Bresson, Jan Słupski, Magdalena Boguta, Małgorzata Cieśla

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The highly conserved protein Maf1 regulates tRNA synthesis by controlling RNA polymerase III (Pol III) transcription initiation. In budding yeast, maf1-deficient (maf1Δ) cells accumulate increased levels of tRNA precursors (pre-tRNA). Because of the saturation of downstream pathways, pre-tRNAs are incompletely processed and hypomodified or fail to be appropriately delivered to ribosomes in maf1Δ cells. Changes in the pool of mature, fully-functional tRNAs presumably disrupts efficient mRNA translation, but this has not been directly tested.

Here, we show that overexpression of the translation elongation factor eEF-1α (TEF1/TEF2) suppresses both the anti-suppressor phenotype and the respiratory defects of the maf1Δ strain. By following 35S incorporation in vivo, we show that protein synthesis is decreased in maf1Δ cells but restored by overexpression of eEF-1α. These differences in translation between strains correlate with growth rate alterations. Notably, maf1Δ cells are hypersensitive to the translation elongation inhibitor cycloheximide (CHX), further supporting a role for Maf1 in translation elongation. By contrast, maf1Δ

cells showed increased resistance for the translation initiation inhibitor lactimidomycin (LTM), though the basis for this is still unclear. Overexpression of eEF-1α reduced the sensitivity of maf1Δ to CHX but did not change the resistance to LTM. We also observed specific changes in mitochondrial translation in maf1Δ cells. De novo levels of mitochondrially-encoded proteins are changed while the corresponding mRNAs did not differ significantly between mutant and wild-type cells. Finally, polysome profiling revealed reproducible changes (elevated level of 40S/60S ratio) in the maf1 deletion mutant which may indicate a potential defect in ribosome synthesis.

Taken together, our results support the hypothesis that MAF1 deletion has multiple effects on translation. We speculate that an imbalance in levels of individual tRNAs adversely affects translation in maf1Δ cells, and eEF-1α overproduction compensates for this defect by facilitating codon adaptation to different tRNA pools. To further characterize the relationship between Maf1 and translation, we are currently applying tRNA-seq and ribosome profiling. Collectively, these studies will illuminate the mutual influence of the tRNA pool and levels of the elongation factor on the rate and specificity of translation for specific mRNAs.

This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012], [UMO-2022/45/N/NZ1/02579] and [UMO-2018/29/B/NZ1/01728].

**38**

## Regulation of the RNA polymerase III transcription machinery is dependent on the ubiquitin ligase Rsp5

**Aleksandra Łopusińska**, Michał Tys, Magdalena Boguta, Małgorzata Cieśla

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Rsp5, a conserved HECT domain-containing E3 ubiquitin ligase, is a multifunctional enzyme that is involved in various signaling pathways, thereby regulating many cellular processes, including RNA metabolism. Most explored is the role of Rsp5 in the ubiquitination and degradation of transcribing RNA polymerase II (Pol II) when stalled on DNA damage. The link between Rsp5 ligase and Pol III has been identified by large-scale proteomics studies, implicating several subunits of Pol III and its general factors, TFIIIB and TFIIIC, as potential substrates for Rsp5. The effect of Rsp5 on Pol III regulation is unknown. We report that the yeast ubiquitin ligase Rsp5 impacts the function of Pol III transcription machinery. The inactivation of Rsp5 leads to the accumulation of newly synthesized tRNAs, reflecting Pol III upregulation. Furthermore, inactivation of the catalytic activity of Rsp5 showed that a decrease in the interaction between the transcription factors TFIIIB and TFIIIC correlates with an increase of Pol III occupancy on tRNA genes. We also detected ubiquitinated forms of the TFIIIC subunit, Tfc3, and demonstrated that the WW3 domain of Rsp5 is essential for interaction with Tfc3. These findings suggest that Rsp5 appears to strengthen the TFIIIB-TFIIIC interaction through the ubiquitination of Tfc3.

This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012]

**39**

## Microtranscriptome of skeletal muscles is regulated by MBNL splicing factors

**Agnieszka Piasecka**, Michał Sekrecki, Arkadiusz Kajdasz, Michał W. Szcześniak, Krzysztof Sobczak

The muscleblind-like proteins (Mbnls) regulate various RNA-processing steps including alternative splicing, polyadenylation, RNA stability, and mRNA intracellular localization. Their sequestration and functional knock-down in myotonic dystrophy type 1 (DM1) lead to the disruption of Mbnls-regulated processes and frequently to the multiple features of DM1. Besides abnormal alternative splicing, disrupted mRNA translation, and mRNA stability, microRNAs (miRNAs) deregulation is observed in the muscles of DM1 patients. miRNAs expression, similar to the regulation of protein-coding genes, is regulated at both transcriptional and post-transcriptional levels. We asked the question of whether Mbnl can be one of miRNAs regulators and whether its depletion results in miRNA level changes.

To address this issue, we performed deep sequencing of a small RNA fraction isolated from Mbnl knock-out mouse model as well as from DM1 mouse model (HSALR). We identified miRNAs sensitive to MBNL depletion and described the mechanisms underlying some changes in microtranscriptome composition via regulation of pri-miRNA splicing. Finally, we compared these results with miRNA data from DM1 patients and showed many similarities in microtranscriptome defects in skeletal muscleles of DM1 patients and mouse models of DM. Moreover, we linked expression alternations of Mbnls during postnatal development with miRNAs level changes occurring during this process. The results revealed a group of miRNA potentially regulated by Mbnl1. Our data provide a key resource for studies of the involvement of Mbnl1 sensitive miRNAs in skeletal muscle development.

---

**40**

## Transcriptome-wide analysis of yeast RNA secondary structures

**Ewa Tykwińska**, Angelika Andrzejewska-Romanowska, Paweł Śledziński, Julita Gumna, Katarzyna Pachulska-Wieczorek

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Knowledge of the native mRNA structure in the cellular environment is necessary to reach a comprehensive understanding of the role of mRNA architecture in the regulation of biological processes. Despite intensive studies, it remains unclear how RNA secondary structure is regulated in vivo and what factors are involved in the remodeling of RNA in the cell.

Here, we present a transcriptome-wide analysis of RNA structure in S. cerevisiae. We used the selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) strategy to explore mRNA secondary structure in living cells. We obtained high-quality data for thousands of yeast transcripts. Comparative analysis between in vivo and ex vivo data revealed a large RNA group whose structure differs significantly across conditions while the others remain unchanged. We also searched for characteristic structural patterns of coding and untranslated regions. We predicted accurate experimentally-supported structure models for S. cerevisiae mRNAs. Our data allow us to analyze the

relationship between the level of the RNA structural complexity and parameters such as translation efficiency and mRNA stability.

This study was supported by the National Science Centre, Poland (project no 2020/39/B/NZ3/03020).

**41**

## Functional suppression of maf1 deletion mutant of Saccharomyces cerevisiae by overdose of N-terminal domains of the largest Pol III subunit, C160

**Malak Farhat**, Aleksandra Łopusińska, Ewa Leśniewska, Małgorzata Cieśla, Magdalena Boguta

Laboratory of tRNA Trascription, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Maf1 is a general and global negative regulator of Pol III transcription. Under repressive conditions Maf1 sequesters Pol III elements involved in transcription initiation and binds the mobile WH2 domain of C34 subunit of Pol III, sealing off the active site (Vorländer et al, 2020).

To further understanding of Pol III regulation, we searched for genetic bypass suppressors of a maf1 deletion mutant (maf1Δ) of Saccharomyces cerevisiae. Strains carrying maf1Δ are temperature sensitive on media containing nonfermentable carbon sources and show the antisuppressor phenotype. Suppressors allowed colonies to grow at the restrictive temperature on glycerol media and partially complemented the antisuppressor phenotype of maf1Δ (Boguta et al., 1997). Second site suppressors of maf1Δ carrying additional chromosomal cold sensitive suppressor mutation mapped in genes encoding C160 or C128 subunits of Pol III and at least one of them showed defect in assembly of Pol III complex (Cieśla et al., 2015).

The DNA plasmids identified as overdose suppressors encoded N-terminal fragments of C160 of various length. The shortest N-terminal fragment of C160, overdose of which partially suppressed the phenotype of antisuppression and temperature sensitive respiratory growth of the maf1Δ, has been named C160-NTF. Plasmid-encoded C160-NTF is expressed in living yeast as a polypeptide of 235 residues. Overdose of C160-NTF leads to lower tRNA levels in the maf1Δ mutant cells documenting functional suppression. Levels of a newly synthetized individual0 tRNAs and Pol III occupancies on tRNA genes are decreased by C160-NTF to various extents. Importantly this negative effect is not related to a defective Pol III assembly because the overdose of C160-NTF does not change the levels and interaction between Pol III subunits. Instead, we propose that Pol III elements involved in transcription activation by interactions with N-terminal domains of native C160 are titrated by overproduced C160-NTF polypeptide.

This work was supported by the National Science Centre [UMO-2019/33/B/NZ1/01012] and [UMO-2021/43/O/NZ1/02291].

**42**

## Detection of non-adenosine residues within poly(A) tails using Oxford Nanopore direct RNA sequencing & deep learning

**Natalia Gumińska**, Katarzyna Matylla-Kulińska, Wiktoria Orzeł, Paweł Krawczyk, Michał Maj, Seweryn Mroczek, Andrzej Dziembowski

Laboratory of RNA Biology, International Institute of Molecular and Cell Biology in Warsaw, Poland

Traditionally, poly(A) tails were thought to be homogenous adenosine stretches. However, nowadays it became clear that their nucleotide composition is more diverse than previously assumed. Numerous enzymes can decorate poly(A) tails with non-adenosine residues, which affects mRNA stability and translation.

All commonly used protocols for detecting non-adenosine residues in poly(A) tail rely on the analysis of the synthetic proxy (cDNA) rather than the direct investigation of the molecules of interest. Oxford Nanopore direct RNA sequencing (DRS) is a promising alternative to the PCR-based methods, since it is immune to amplification bias and delivers an accurate, ground-truth reflection of the native RNAs within the sample, yet there is currently no computational method for detecting composite poly(A) tails in DRS data.

To fill this gap, we developed the Ninetails – a swiss-army tool for detection and profiling of non-adenosines within poly(A) tails. Our software performs an angular transformation of the raw signal followed by classification using a convolutional neural network trained on a large number of synthetic RNA molecules. We leveraged the Ninetails to analyze various biological contexts in which mixed tails were assumed to play a role in post-transcriptional gene regulation. Herein, the Ninetails package and the results of the functional analysis are demonstrated.

---

**43**

## Discovering structural features of the active Ty3 retrotransposon RNA genome

**Angelika Andrzejewska-Romanowska**, Julita Gumna, Ewa Tykwińska, Katarzyna Pachulska-Wieczorek

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

Long terminal repeat (LTR)-retrotransposons are non-infectious mobile genetic elements widespread in eukaryotic genomes. The Ty3/Gypsy elements constitute an important and representative family of retrotransposons and are considered progenitors of retroviruses. Functional Ty3 element contains two partially overlapping ORFs: GAG and POL flanked by LTRs. A vast majority of retrotransposons have lost the ability to mobilize, but their sequences have been domesticated and code proteins used in various biological pathways of the host cells. In the human genome, a large group of adapted sequences is derived from the GAG gene of the Ty3/Gypsy retrotransposons.

Ty3 replicates via an RNA intermediate, which serves as mRNA for element-encoded proteins and genomic RNA (gRNA) that is reverse-transcribed in virus-like particles. The resulting cDNA can integrate into the host genome, thereby duplicating the element. Here, we present the first secondary structure model of the entire RNA genome (5.2 kb) of the Ty3 retrotransposon replicating in yeast. In brief, using the high-throughput SHAPE-MaP (selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling) method, we explored Ty3 gRNA structure in the in vivo and ex vivo states to analyze retrotransposon gRNA structural dynamics. We also analyzed RNA-RNA interactions essential for retrotransposition, which in the host cell are induced by Ty3-encoded Gag chaperone protein. Our data contribute to a better understanding biology of complex and multifunctional RNAs.

This study was supported by the National Science Centre, Poland (project no 2019/35/N/NZ1/01954 and 2021/41/N/NZ3/04060).

**44**

## Identification of proteins involved in the regulation of double-stranded RNA level in the nucleus of human cell

**Karolina Kasztelan**, Aleksander Chlebowski, Tomasz M. Kuliński, Kamila Affek, Andrzej Dziembowski, Roman J. Szczęsny

International Institute of Molecular and Cell Biology in Warsaw, Poland

The presence of double-stranded RNA (dsRNA) in a cell is most frequently related to a viral infection. However, there are an increasing number of studies which demonstrate the existence of endogenous sources of dsRNAs, which are associated with both physiological processes and cellular dysfunctions. Under normal conditions in human cells, endogenous dsRNA molecules with double-stranded stretches longer than 30 base pairs can be detected almost exclusively in mitochondria as a product of bidirectional transcription of mitochondrial genome, meanwhile in the nucleus the amount of long dsRNAs fluctuates at a very low and almost undetectable level. Therefore, to understand control mechanisms of dsRNA level in the nucleus, we performed a genome-wide loss-of-function screen. Depletion of several nuclear proteins resulted in a strong accumulation of dsRNA in the nucleoplasm of HeLa cells. Interestingly, many of identified proteins are functionally interrelated and additionally are proved to be physically connected with mitotic kinetochore. Our data show that the observed increase of dsRNA levels is strongly related to the cell cycle. Preliminary data of deep sequencing of immunoprecipitated dsRNAs revealed significant enrichment in RNAs derived from repetitive elements, as well as such phenomena as transcriptional readthrough, transcription using alternative initiation sites, convergent transcription and transcription from centromeric regions. This suggests the presence of global transcriptional dysregulation upon depletion of identified proteins leading to the formation of abnormal RNAs potentially containing fragments capable of forming duplexes. Our progress toward understanding the molecular basis of dsRNA accumulation and its physiological consequences will be presented.

45

## The role of the ProQ protein in RNA-RNA interactions involving small RNAs in Escherichia coli

**Joanna Zwolenkiewicz** and Mikołaj Olejniczak

Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

Bacterial regulatory RNAs (sRNAs) base pair with complementary sequences in mRNA targets, which leads to changes in their translation or stability. sRNAs are often assisted by RNA-binding proteins. Among them is a chaperone protein Hfq, which promotes sRNA-mRNA interactions, and FinO-domain proteins, such as ProQ protein, which binds numerous RNAs in Escherichia coli. ProQ and Hfq bind mostly distinct pools of RNAs in E. coli. Global profiling studies showed that different RNAs can simultaneously bind to ProQ, but it is not clear if ProQ contributes to RNA-RNA pairing.

To explore this question we compared how ProQ affects the kinetics of RNA-RNA complex formation for two RNA pairs: cis-encoded sRNA SibA pairing with ibsA mRNA, and trans-encoded sRNA RybB with sponge RNA 3'ETSLeuZ. The association progress was monitored using a gelshift assay, and analyzed using KinTek Explorer software. The data showed that the formation of the complex of SibA with ProQ was kinetically preferred, but this fast-formed SibA-ProQ complex was replaced over time by the more thermodynamically stable SibA-ibsA complex. On the other hand, ProQ remained bound to both RybB sRNA and 3'ETSleuZ RNA after their pairing. However, further mutational study suggested that ProQ binding to one of these RNAs slows RNA-RNA pairing, which could reflect overlap between the ProQ binding site and the RNA pairing site. These results suggest that the role of ProQ in interactions between complementary RNAs may depend on the relative orientations of ProQ binding sites and RNA pairing sites.

---

46

## TimeSeqR: a comprehensive R package for integrated analysis of transcriptomic and translatomic data from time-course RNAseq and RIBOseq experiments

**Przemysław Latoch**, Olga Iwańska, Mariia Kovalenko, Agata Starosta

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

The rapid advancements in next-generation sequencing (NGS) technologies have transformed molecular biology, enabling more precise, faster, and cheaper sequencing. These technologies have led to a vast amount of data and increased computational demands, necessitating the development of efficient and integrated approaches to analyze and interpret this information. We present TimeSeqR, an R package designed to address the gap in integrated analysis for time-course RNAseq and RIBOseq data.

TimeSeqR enables simultaneous utilization of data from parallel RNAseq and RIBOseq experiments, allowing for the identification of genes with similar or distinct expression patterns over time following an induced change. This comprehensive approach facilitates more accurate investigation of factors

influencing genomic information utilization within both the transcriptome and the translatome. TimeSeqR provides a complete solution for time-course RNAseq and RIBOseq data analysis, encompassing various aspects such as data exploration through heatmaps, identification of differentially expressed genes with ImpulseDE2, and gene expression clustering in time using the fuzzy c-means algorithm implemented in Mfuzz. Furthermore, TimeSeqR supports functional analysis of the obtained gene clusters through gene ontology enrichment. To enhance user experience, TimeSeqR features a user-friendly graphical interface built upon the R shiny library, allowing for seamless interaction with the provided analytical tools. By offering an integrated approach to transcriptomic and translatomic data analysis, TimeSeqR aims to advance our understanding of the complex processes governing gene expression and translation regulation in living organisms, ultimately contributing to the progress of molecular biology research.

---

**47**

## Structure of the human tRNA methyltransferase complex FTSJ1-THADA

**Alexander Hammermeister**, Agnieszka Broniec, Sebastian Glatt

Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

tRNAs are extensively post-transcriptionally modified at various positions in their body. A modification hotspot is the anticodon stem loop (ASL). Modifications in the ASL are predominantly involved in the translational decoding process and lack of it is associated with various human diseases. The conserved human tRNA 2'-O-methyltransferase (MTase) FTSJ1 is responsible for the methylation of the ribose 2'-OH moity of residues 32 and 34 in the ASL of a subset of tRNAs. In order to do so, FTSJ1 forms a complex with THADA to methylate residue 32 and with WDR6 for residue 34. Further, it was reported that mutations in FTSJ1 are linked to X-linked intellectual disability and cancer. Here, we present the first cryo-EM structure of FTSJ1-THADA complex in its apo, tRNA and cosubstrate bound form. Our structure reveals that THADA binds tRNA in its cavity and positions the ASL in the vicinity of the FTSJ1 active site. We can observe that the ribose 2'-OH group of residue 32 points towards FTSJ1 and the methyl donor S-adenosyl-L-methionine is in close proximity. Additionally, we noticed that several residues of THADA are involved in base stacking interaction with tRNA, which could explain the specificity of FTSJ1 to its target tRNAs.

---

**48**

## Elucidating the molecular mechanisms underlying the synthesis of sdRNAs mediated by FUS

**Patrycja Świergiel**

Adam Mickiewicz University, Poznań, Poland

Small nucleolar ribonucleoproteins (snoRNP) are nucleolus-localized complexes involved in rRNA modifications, that consist of small nucleolar RNAs (snoRNAs) and associated core proteins. A growing amount of evidence indicates that snoRNAs are processed into shorter, stable and functional molecules, called sdRNAs - small RNAs derived from snoRNAs. However, the mechanism of sdRNA

generation is still not completely understood. Recently, by RNA immunoprecipitation followed by high-throughput sequencing (RIP-seq), Fused in Sarcoma (FUS) protein was found to bind snoRNAs in human cells. Further analysis revealed that FUS negatively regulates the level of mature snoRNAs. FUS might compete with snoRNP proteins and induce the synthesis of sdRNAs, which in turn, leads to a decreased level of mature snoRNAs. The main goal of the project is to elucidate the molecular mechanisms of sdRNAs synthesis mediated by FUS.

During the conference, I will present the results of RNA antisense purification (RAP) of two selected endogenous, differentially expressed snoRNAs with corresponding, stably expressed, control snoRNAs using biotinylated complementary oligonucleotides and streptavidin magnetic beads, followed by protein identification by mass spectrometry. Experiments were performed in SH-SY5Y cells with FUS knockout in comparison to wild-type cells. Detailed analysis of the pulldown protein fraction will enable to distinguish proteins that are recruited to the snoRNP complex by FUS.

**49**

## Impact of the bunyaviral mRNA 3'UTR on transcription and translation

**Eliana Dukhno**, Moh Egy Rahman Firdaus, Piotr Gerlach

IMol, Polish Academy of Sciences, Warsaw, Poland

Bunyaviruses are a huge group of viruses with segmented, negative-sense RNA genome, that are not well-studied so far. Bunyaviral mRNA contains host-derived, short 5'capped oligo, and interestingly does not have a poly(A) tail. Working on a model Bunyamwera virus, Barr et al. showed in 2006 that bunyaviral mRNA has structural features in the 3'UTR end, which are required for transcription termination. Later in 2009, Blakqori et al. described that a stem-loop in the Bunyamwera mRNA 3'UTR is important for the viral translation. In our studies, we use La Crosse (LACV) and Bunyamwera (BUNV) orthobunyaviruses to explore the mechanistic role of the 3'UTR end in transcription termination and translation regulation. We use an in vitro transcription assay with purified LACV polymerase to test how modifications of predicted LACV mRNA 3'UTR stem-loop affect the transcription termination, thus leading to the formation of different length mRNAs. We explore as well, how the same stem-loop modifications affect translation efficiency of the LACV mRNA. To do so, we transfect T7-transcribed mRNA constructs coding for renilla luciferase reporter, flanked by the LACV mRNA UTRs, and we monitor the translation efficiency. Finally, we want to identify host factors that bind to the LACV mRNA 3'UTR and are thus involved in the regulation of the bunyaviral translation.

**50**

## Mapping Active Transcription Sites of Human RNA Polymerase III at Nucleotide Resolution

**Jan Mikołajczyk** and Tomasz W. Turowski

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Eukaryotic organisms rely on RNA polymerase III (RNAPIII) to produce 5S rRNA, tRNAs, U6 snRNA, and other small untranslated RNAs. Unlike RNAPII, which transcribes long genes, RNAPIII transcribes relatively short units of 75-300 nucleotides in length and possesses intragenic promoters.

In this study, we used UV cross-linking and analysis of cDNA (CRAC) to precisely map actively transcribing RNAPIII with high resolution and strand specificity in a human leukemia cell line K562. By leveraging the high signal-to-noise ratio of CRAC, we identified the transcription start sites (TSS) of RNAPIII units and elucidated the kinetics of the transcribing polymerase.

We observed high variability in transcription rate between tRNA genes. At the transcriptional unit level, we detected uneven coverage, suggesting nonuniform RNAPIII kinetics. Interestingly, RNAPIII showed a 3' bias on tRNA genes, which is in marked contrast to RNAPI and RNAPII that present a 5' transcriptional bias. In intron-containing tRNA genes, RNAPIII occupancy was also significantly reduced in the intronic regions, which we interpreted as an increased elongation rate upon transcription through tRNA introns. Consistent with findings in yeast, we also detected widespread read-through of termination signals by human RNAPIII.

Additionally, we discovered transcription in previously unannotated genomic regions. Our initial results reveal new aspects of RNAPIII transcription kinetics and contribute to the understanding of the expression of the human genome.

---

**51**

## Chloroplast retrograde control of miRNA expression in response to high light stress

**Anna Barczak-Brzyżek**, Grzegorz Brzyżek, Marek Koter, Ewa Siedlecka, Piotr Gawroński, Marcin Filipecki

Warsaw University of Life Sciences, Poland

In response to developmental and environmental cues such as high light (HL), chloroplasts produce and emit signals which affect the expression of nuclear genes. This signaling process is called retrograde communication. We show that HL is involved in miRNA-dependent regulation in Arabidopsis thaliana rosettes and roots. Microtranscriptomic screening revealed a limited number of miRNAs reacting to HL. To explain the miRNA regulation in HL chemical and genetic approaches were applied. First, we tested the possible role of plastoquinone (PQ) redox changes using photosynthetic electron transport chain inhibitors. The results suggest that increased primary transcript abundance (pri-miRNAs) of HL-regulated miRNAs rely on signals upstream of PQ. It pointed the source of regulatory signal is photosystem II, which produces singlet oxygen ($^1O_2$). No changes in pri-miRNA expression upon a

dark–light shift in the conditional fluorescent (flu) mutant accumulating 1O2 were observed compared to wild-type plants. Thus, we explored the 1O2 signaling pathway initiated independently in HL and related to β-carotene oxidation and the production of its volatile derivatives, such as β-cyclocitral (β-CC). Since β-CC caused an increased level of pri-miRNAs and the methylene blue sensitivity 1 (mbs1) mutant represents an altered miRNA response to HL, it supports the role of 1O2-β-CC signaling in miRNA regulation. Moreover, inconsistency between the pri- and mature miRNA levels in HL conditions pinpointed the importance of post-transcriptional mechanism of miRNA control.

**52**

## miR-30a conjugated with cholesterol as a novel therapeutic approach in the treatment of rhabdomyosarcoma

**Paweł Konieczny**[1], Mirosław Cebula[2], Marta Kot[1], Klaudia Skrzypek[1], Marcin Majka[1]

[1] Department of Transplantation, Institute of Pediatrics, Jagiellonian University Medical College, Kraków, Poland

[2] Faculty of Biotechnology and Horticulture, University of Agriculture, Kraków, Poland

SNAI1 is a transcription factor that regulates the repression of E-cadherin during the epithelial-mesenchymal transition (EMT), a process that enables epithelial cells to acquire a mesenchymal phenotype. While EMT is critical in embryogenesis and tissue repair, its dysregulation has been implicated in the development and progression of various tumors, including rhabdomyosarcoma, the most common soft tissue sarcoma in children. Current therapies for rhabdomyosarcoma remain suboptimal, highlighting the need for novel therapeutic strategies.

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression by binding to target mRNAs. Among the miRNAs that have been implicated in EMT and tumorigenesis is the miR-30 family, which has been shown to negatively regulate the expression of SNAI1. The present study aims to explore the potential of miR-30a, a member of the miR-30 family, as a therapeutic agent for rhabdomyosarcoma.

Our results showed that Chol-miR-30a had a higher uptake efficiency of about 25% compared to unconjugated miR-30a. In addition, Chol-miR-30a was more effective in downregulating SNAI1 expression in rhabdomyosarcoma cells. We also evaluated the intracellular stability and retention of miR-30a, which confirmed sustained therapeutic efficacy.

In conclusion, our findings provide valuable insight into the potential of miR-30a as a novel therapeutic approach for rhabdomyosarcoma treatment and facilitate the development of lipoprotein-conjugated miRNA delivery systems for other types of cancer.

This work was supported by research grant 2018/29/B/NZ5/00915 from the National Science Center in Poland.

**53**

## Epigenetic control of transcription during keratinocyte differentiation

**Bart Krist**, Tansu Doran, Katarzyna Czerniak, Yaarob Altalli, Michal Gdula

Adam Mickiewicz University, Poznań, Poland

One of still not fully understood aspects of epidermal differentiation is the importance of changes in the chromatin higher-order structure for epidermis-specific gene regulation. Big part of the genes involved in epidermal differentiation are clustered in several loci. This clustering is important for establishment of their proper spatio-temporal expression pattern. Previous work showed that one of those loci, epidermal differentiation complex (EDC), harboring almost 70 of genes, relocates during development from nuclear periphery to the interior.

We are investigating how the changes in the architecture of the epidermal chromatin domains affects promoter-enhancer networks and how it leads to establishment of keratinocyte-specific transcription throughout the development. Our re-analysis of published RNA-seq, ATAC-seq data shows high degree of similarity between the mouse and human keratinocytes. It also demonstrates different levels of temporal coordination between genes located in distinct parts of the EDC, Ker I and Ker II loci.

---

**54**

## The transcriptomic basis of rhizomania resistance in sugar beet

**Gabriela Machaj**, Dariusz Grzebelus, Guillem Ylla

Laboratory of Bioinformatics and Genome Biology, Faculty of Biochemistry, Biophysics

Rhizomania is a severe disease that affects sugar beets. It is caused by the Beet Necrotic Yellow Vein Virus and produces high-yield losses. Currently, the control of the diseases is through the usage of varieties that are tolerant or resistant to the virus. Resistance genes (e.g., Rz1, Rz2) were identified in wild relatives and introduced to cultivated varieties. The molecular mechanisms of action of the resistance genes remain unknown.

To identify the molecular mechanism by which the Rz1 genes induce resistance against rhizomania, we performed a comprehensive comparative transcriptomic analysis of resistant (Rz1/Rz1) and susceptible (rz1/rz1) plants. In this analysis, we look at both, the role of mRNAs as well as that of small RNAs, and how they can interplay to protect the plant from such a severe virus.

In total, we generated 22 mRNA-seq and 12 smallRNA-seq libraries from resistant and susceptible plants, in virus-free and virus-contaminated soils. A thorough, comparative analysis of mRNA and small RNA between different phenotypes and conditions allowed us to identify protein-coding genes and small RNAs involved in rhizomania resistance.

Overall, in this project, we leverage a comprehensive transcriptomic dataset to get insights into the molecular basis of rhizomania disease in sugar beet. At the same time, our findings could have a broader impact, given that other plants might use similar mechanisms to protect against other viruses.

The research was financed by the Polish Ministry of Agriculture and Rural Development and the Polish National Science Center (MINIATURA6).

**55**

## Endogenous tuning of MBNL1 corrects alternative splicing defects in a cell model of myotonic dystrophy type 1 (DM1)

**Nikola Musiała** and Ewa Stępniak-Konieczna

Adam Mickiewicz University, Poznań, Poland

One of the key molecular hallmarks of myotonic dystrophy type 1 (DM1) is sequestration and functional depletion of Muscleblind-like (MBNL) proteins by DMPK (DM1 Protein Kinase) transcripts carrying expanded CUG-triplet repeats (CUGexp RNA). MBNLs are master regulators of cellular RNA metabolism and one of their key functions involves regulation of alternative splicing (AS) events. In DM1, sequestration of MBNL proteins on nuclear foci formed by CUGexp RNA impairs their function, shifting the AS pattern of hundreds of target RNAs from adult to fetal splice isoform. Proteins translated from these mis-spliced mRNA isoforms are functionally unsuitable in an adult organism, thus leading to a plethora of pathological hallmarks including myotonia, muscle atrophy and wasting, insulin resistance and cataract, among others. In this work, we employed targeted approach based on transcriptional stimulation of the MBNL1 gene via RNA activation (RNAa). This evolutionarily conserved mechanism allows to increase gene expression by small activating RNA (saRNA) complementary to specific gene promoter sequences. We have designed and screened a series of saRNA duplexes targeted to the most active MBNL1 promoter 2 (P2). Our initial analyses identified two saRNA sequences that strongly upregulated MBNL1 transcription and protein levels in patient-derived DM1 cell model. Importantly, identified saRNA molecules corrected the alternative splicing of several tested MBNL1 target pre-mRNAs in DM1 cells. Taken together, this novel approach demonstrates the therapeutic utility of RNA-based antisense mechanism in the treatment of DM1 via targeted upregulation of MBNL1 expression.

**56**

## Role of SmcHD1 in the transcription inactivation and replication timing shift on female X chromosome

**Anna Piaszyk-Borychowska**, Kamil Wójcik, Katarzyna Czerniak, Michał Gdula

Adam Mickiewicz University, Poznań, Poland

X chromosome inactivation is the mechanism that evolved in mammals to equalize levels of X-linked gene expression in XX females relative to XY males. Cells of early female embryos selectively inactivate a single X chromosome, usually at random, resulting in the formation of a stable heterochromatic structure, the Barr body. The inactive X chromosome (Xi), once established, is highly stable, and is maintained in somatic cells throughout the lifetime of the animal1. The X inactivation process is triggered by the non-coding RNA Xist, which localizes to the Xi territory to induce chromosome-wide gene silencing. Chromatin features that distinguish Xi and the active X chromosome (Xa) include specific histone post-translational modifications, variant histones and CpG DNA methylation.

Furthermore Specifically, A-type chromatin compartments, corresponding to gene-rich regions which normally replicate in early S-phase, switch to replication in mid- or late-S-phase.

Our bioinformatic and 3D image analysis characterizes organization of transcription and DNA replication on X chromosome in wild-type and SmcHD1 KO cells at different stages of development. Our results shed the light how both processes are interlinked.

---

**57**

## Structural and functional characterization of the human tRNA thiolation cascade

**Marta Walczak**[1,2] and Sebastian Glatt[1]

[1] Jagiellonian University, Malopolska Centre of Biotechnology, Max Planck Research Group, Kraków, Poland

tRNA modifications are essential for the proper and efficient functioning of protein synthesis. To date, more than 100 tRNA modifications have been identified. The exchange of oxygen with sulfur (thiolation) at the C2 position of wobble base uridines (U34) facilitates codon recognition, reading frame maintenance and accurate protein synthesis. In humans, the highly conserved CTU1-CTU2 complex carries out the final reaction step and modifies 4 tRNAs, namely tRNA Lys(UUU), tRNA Glu(UUC), tRNA Gln(UUG) and tRNA Arg(UCU). However, the exact mechanism of the CTU1-CTU2-mediated sulfur transfer reaction and the requirements for tRNA recognition as well as selectivity remain elusive. Thiolation of tRNAs is clinically relevant and it is crucial for the proper functioning of cells as well as organisms. The lack of uridine thiolation disrupts cellular protein homeostasis and leads to occurrence of various neurodegenerative disorders.

Project aims to investigate and explain the mechanism of tRNA thiolation in humans and other eukaryotes at the molecular level. We heterologously produced the CTU1-CTU2 complexes from different organisms in insect cells and purified them to homogeneity. With the use of single particle cryogenic electron microscopy (cryo-EM) and complementary biochemical approaches, we now seek to understand the mechanism of the final step of the thiolation cascade. In detail, we aim to characterize the binding specificity of different tRNA substrates, to image different reaction intermediates at high-resolution and to identify the role of additional binding partners.

---

**58**

## Arabidopsis Era1 GTPase homolog is involved in 16S rRNA maturation in chloroplasts

**Kinga Gołębiewska** and Piotr Gawroński

Warsaw University of Life Sciences, Poland

Chloroplasts are semi-autonomous organelles that originated from endosymbiosis and share many characteristic features with prokaryotic molecular systems. The regulation of the chloroplast gene expression primarily occurs during translation and requires various factors that are encoded predominantly by the nuclear genome. However, all necessary rRNAs and tRNAs, that constitute the chloroplast translation machinery are encoded by chloroplast genome (plastome).

One of the proteins, that plays a role in rRNA maturation and contributes to the assembly of the small (30S) ribosomal subunit is Era1 GTP-ase. This protein stimulates 16S rRNA maturation by binding to the anti-Shine-Dalgarno region followed by GTP hydrolysis, which signals for RNazes recruitment. The aim of this study was to initially characterise a novel protein in Arabidopsis thaliana, the Era1 putative homolog encoded by the Era1 gene.

The results indicate that the Era1 protein is localized in chloroplasts, likely in nucleoid regions. The Arabidopsis era1 mutants accumulate precursors of 16S rRNA as confirmed by qRT-PCR, cRT-PCR and RACE. Additionally, these mutants exhibit a negative influence on the function of the photosynthetic apparatus and inhibition of photosynthetic pigments accumulation. Moreover, era1 mutants display inhibited chloroplast translation following treatment with lincomycin and chloramphenicol.

Thus, our findings suggest that Arabidopsis Era1 homolog is an important factor in chloroplast translation, playing a role in 16S rRNA maturation.

---

**59**

## Spatial transcriptomics of mouse pituitary gland

**Ewelina Kałużna**, Julian Zacharjasz, Monika Piwecka

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

In our laboratory, we are interested in non-coding RNAs that regulate gene expression in pituitary gland (PG) cells, especially in the context of hormone production and hormone release. We used spatial transcriptomics (ST) to map polyadenylated transcriptome within the adult mouse PG to better understand the spatial distribution of RNA within PG anatomical regions, its endocrine and non-endocrine cell types, and cell-to-cell topology. We used Visium technology and evaluated 12 PG sections. It allowed us to capture transcripts representative of >21,900 genes, with a median of >6,000 genes per sample. Based on dimensionality reduction (tSNE) and marker gene analysis, we were able to map 9 clusters of spots that represented different anatomical regions and consequently, enriched for the cell types characteristic to these regions. These data are a good resource for in-depth studies of the intermediate lobe and posterior lobe within PG and their gene expression programs. For example, we found good coverage for neural mRNAs in the posterior lobe that are representative of transcripts

being transported from the hypothalamus to PG in axons of magnocellular neurons. Similarly, we transcriptionally mapped stem cell niches in PG. Currently, we are working on the integration of ST data with single-cell RNA sequencing data in order to get better insight into the anterior lobe of PG, which is most densely packed with cells and where individual Visium spot covers a few different hormone-producing cells. Moreover, we are moving on to analyzing polyadenylated long non-coding RNAs in these datasets.

---

**60**

## Superior properties of mRNA capped with N2 modified dinucleotide cap analogs

Renata Grzela, **Karolina Piecyk**, Anna Stankiewicz-Drogon, Paulina Pietrow, Maciej Lukaszewicz, Karol Kurpiejewski, Paulina Skrzypczyk, Eliza Rajkowska, Edward Darzynkiewicz, Marzena Jankowska-Anyszka

University of Warsaw, Poland

One of the basic structural elements in mRNA that are required to keep mRNA functional and stable is a 5'-end cap structure that consists of 7-methylguanosine connected via 5'-5' triphosphate bridge to the first transcribed nucleoside. This unique structure is recognized by highly specialized cap-binding proteins, and is involved in a number of processes, including maturation, nuclear export, initiation of translation and turnover of mRNA. A lot of in modified cap analogs has been observed in recent years due to the fact that they can be used as a part of therapeutic mRNAs. One of the first modified cap analog that was used in order to achieve correct orientation during in vitro transcription reaction incorporation of chemically synthesized cap analog into mRNA were Anti-Reverse Cap Analogs (ARCA) possessing the methyl group at the C2' or C3' position of 7-methylguanosine. This type of analogs have also been used in current mRNA vaccines against COVID. Within this work we describe the continuation of our previously published research with the use of a wider range of cap analogs modified within the exocyclic amine group of 7-mrthylguanosine and/or with extended phosphate chain. The conducted research concerns both the analysis of cap orientation of obtained capped RNA and their translational properties in RRL and HEK293 cells.

---

**61**

## Function of SF3 spliceosome toward function of skeletal muscle progenitors

**Kevork Wakimian**, Vladyslava Liudkovska, Maciej Cieśla

IMol, Polish Academy of Sciences, Warsaw, Poland

The purpose of this research is to understand how the SF3 spliceosome components interact during activation of somatic stem cells. Splicing is an abundant process that enhances proteome diversity by generation of multiple proteins from a single transcript. This process is catalyzed by the spliceosome which consists of 5 catalytic RNAs and over 150 protein splicing factors (SFs) that form small nuclear

ribonucleoprotein complexes (snRNPs). Our study focuses specifically on the core spliceosome complex SF3, which is a central component of the U2 snRNP. While we previously shown that control of SF levels from SF3 plays a crucial role in regulating splicing outcomes, its dynamics during stem cell activation remains unknown. We hypothesize that SF3 spliceosomes are 'tuned' to meet the demand of stem cell quiescence and differentiation.

Toward this end we use skeletal muscle progenitor cells C2C12, that rely on fast cell fate transitions. We analyzed the dynamics of SF3 components during C2C12 differentiation by determining the levels of individual proteins both at transcriptional and protein levels. To study the functional role of each protein from the SF3 complex, we generated tools for their downregulation employing lentiviral vectors harboring short hairpin RNAs.

Our results show dynamic changes in protein expression of selected components of SF3 during differentiation, particularly from SF3b subcomplex. Our data also suggests uncoupling between transcript and protein levels, indicating possibility of translational control of SF3 node upon muscle differentiation.

Collectively, our research provides insights into the dynamics of splicing during somatic stem cell activation and differentiation.

---

**62**

## A comprehensive transcriptome analysis reveals a novel catalog of long non-coding RNAs dependent on driver oncogenes (CMYC, KRAS, mutant p53)

Akanksha Jaiswar, Dawid Walerych, **Maria Grześ**

Laboratory of Human Disease Multiomics, Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland

Although lncRNAs play a crucial role in almost every aspect of cancer biology and are promising therapeutic targets, their functional characterization is still largely unknown. In this study we aimed to identify lncRNAs dependent on three driver oncogenes: mutant KRAS, mutant p53 and hyperactive CMYC, for which no standard clinical protocols for direct targeting are available.

For this purpose we analyzed RNA-seq data obtained by knock-out of above mentioned oncogenes with CRISPR/Cas9 in a panel of 8 colorectal and lung cancer cell lines. We developed an in-silico pipeline for transcript filtering based on their length and coding potential and we assessed the RNA-seq quality with PCA. Differential expression analysis revealed the highest number of mRNAs and lncRNAs with expression dependent on CMYC and the lowest in case of mutant p53, with a proportion of mRNAs and lncRNAs switching oncogene dependence among the studied cell lines. Differentially expressed lcnRNAs were characterized according to their length, chromosome-wise distribution and biotype classification. Coexpression analysis of lncRNAs and mRNAs combined with lncRNAs target prediction revealed several lncRNAs that may be functionally involved in cancer progression. Their expression was measured in colon and pancreatic cancer samples with ddPCR. We also validated oncogenic potential of these lncRNAs using cell lines (normal and cancer) with oncogenes depletion/overexpression as well as analyzing patients expression data.

Our study identified a novel catalog of lncRNAs dependent on the driver oncogenes, providing the groundwork for further research exploiting the molecular mechanisms of lncRNAs interaction with oncogenes for anti-cancer treatment development.

**63**

## miR-125b-5p – a mediator of EV biogenesis and secretion at the embryo-maternal interface

**Maria M. Guzewska**[1], Krzysztof J. Witek[2], Elżbieta Karnas[3], Michał Rawski[4], Ewa Zuba-Surma[3], Monika M. Kaczmarek[1,5]

[1] Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland
[2] Cell and Tissue Analysis and Imaging Laboratory, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland
[3] Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[4] Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland
[5] Molecular Biology Laboratory, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

Cells release extracellular vesicles (EVs) as population of nanoparticles, which cross the cellular barriers and participate in intercellular communication. The EV-mediated exchange of signals at the embryo–maternal interface is considered to be a fundamental element of successful reproduction and promoting embryo implantation. Among various routes of EV biogenesis and trafficking, ESCRT-mediated pathway is responsible for creation of multivesicular bodies. The ESCRT is comprised of an ancient system of 20 proteins that assemble into four subunits. In this study, we sought to analyse the impact of miR-125b-5p, found in the uterine environment during pregnancy, on EV biogenesis and trafficking. We hypothesize that miR-125b-5p affects the expression of ESCRT complexes and consequently disturbs release of EVs.

To test our hypothesis, we used primary trophoblast (pTr) cells collected at day 16 of pregnancy from domestic animal model – Sus scrofa. Bioinformatics tools were used to identify miR-125b-5p targets among members of ESCRT-dependent pathway. miRNA-mRNA binding motifs were found for 3'UTRs of genes – VPS22, VPS25, VPS28, VPS36, VPS37B, Alix, and VAMP8. Cells were transfected with miR-125b-5p what lead to decrease of expression in four genes. Finally, stepwise centrifugation of media collected after treatment with control mimic or miR-125b-5p was performed to characterize secreted EVs. Strikingly, CD63-bearing EVs were more abundantly secreted by pTr cells after miR-125b-5p delivery.

We favour the hypothesis that miRNA presence in uterine environment is a key element of ongoing EV-mediated communication between the mother and developing conceptus, leading to the generation and secretion of specific subpopulations of EVs.

**64**

## MODOMICS: a database of RNA modification pathways

Pietro Boccaletto[1], Filip Stefaniak[1], Angana Ray[1], Andrea Cappannini[1], Sunandan Mukherjee[1], Elżbieta Purta[1], Małgorzata Kurkowska[1], Niloofar Shirvanizadeh[1], Eliana Destefanis[2], Paula Groza[3,4], Gülben Avşar[5], Antonia Romitelli[6,7], Pınar Pir[5], Erik Dassi[2], Silvestro G. Conticello[7,8], Francesca Aguilo[3,4], **Janusz M. Bujnicki**[1]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Department of Cellular, Computational and Integrative Biology, University of Trento, Italy
[3] Department of Molecular Biology, Umeå University, Sweden
[4] Wallenberg Centre for Molecular Medicine, Umeå University, Sweden
[5] Department of Bioengineering, Gebze Technical University, Kocaeli, Turkey
[6] Core Research Laboratory, ISPRO—Institute for Cancer Research, Prevention and Clinical Network, Firenze, Italy
[7] Department of Medical Biotechnologies, Università di Siena, Italy
[8] Institute of Clinical Physiology, National Research Council, Pisa, Italy

Since its inception in 2005, the MODOMICS database has been a valuable, manually curated, centralized repository for comprehensive data on modified ribonucleosides. It originally hosted information on chemical structures, biosynthetic pathways, modified residue locations within RNA sequences, and RNA-modifying enzymes. With the acquisition of new knowledge and novel data types, the database has continually updated its information and functionalities. Most recently, we have expanded MODOMICS to include a catalog of RNA modifications associated with human diseases, specifically those resulting from mutations in genes encoding modification enzymes. We have also established extensive links to the RCSB Protein Data Bank and added sequences of experimentally determined RNA structures featuring modified residues. This extension is complemented by the inclusion of nucleotide 5´-monophosphate residues. Moreover, we have integrated a search engine for chemically similar modified residues that can be queried using SMILES codes or by inputting drawn chemical molecules. Updates have been made to the existing datasets of modified residues, biosynthetic pathways, and RNA-modifying enzymes. In our quest to enhance user experience, we have redesigned the web interface and upgraded the database backend, thereby enabling more flexible representation of sequences with modified residues. Our present endeavors concentrate on augmenting MODOMICS data with annotations regarding relative reliability, a measure that will aid in distinguishing modified residues validated by numerous direct biochemical and biophysical assays, from those extrapolated from mutation signatures in high-throughput sequencing experiments.

MODOMICS can be accessed at https://iimcb.genesilico.pl/modomics/.

References:
Boccaletto et al., MODOMICS: a database of RNA modification pathways. 2021 update. Nucleic Acids Res. 2022 Jan 7;50(D1):D231-D235.18:bbad187.

Dunin-Horkawicz et al., MODOMICS: a database of RNA modification pathways. Nucleic Acids Res. 2006 Jan 1;34(Database issue):D145-9.

**65**

## SimRNA-Cry: a tool for modeling RNA molecules using maps from cryo-electron microscopy

**Sunandan Mukherjee**[1], Masoud A. Farsani[1], Matthias Bochtler[2], Janusz M. Bujnicki[1]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Laboratory of Structural Biology, International Institute of Molecular and Cell Biology in Warsaw, Poland

RNA molecules, as vital cellular regulators, have increasingly gained prominence over the past few decades due to their diverse functions within biological systems. Similar to proteins, RNA function is defined by its structure and conformational adaptability, which are encoded within its sequence. However, experimental determination of precise RNA structures is strenuous and complex, resulting in a large portion of RNAs remaining structurally uncharacterized. To circumvent this issue, computational prediction methods have been conceived, incorporating existing RNA structure knowledge, the fundamentals of RNA folding, and evolutionary aspects such as the preservation of crucial functional motifs. Despite their utility, these theoretical methods are often limited in accurately predicting structures for RNA sequences exceeding 100 nucleotides without supplementary experimental data.

Our laboratory previously developed SimRNA (1), an RNA folding simulation and 3D structure prediction program that uses a coarse-grained molecular representation, the Monte Carlo method for sampling conformational space, and statistical potentials for energy approximation and identification of biologically relevant conformations. Independently benchmarked by RNA-Puzzles and CASP, SimRNA has demonstrated high effectiveness. We recently developed an enhancement, SimRNA-cry, which leverages 3D maps from X-ray crystallography or cryo-electron microscopy. This newe method can effectively build models within high-resolution maps and aid the interpretation of low-resolution data, particularly valuable for cryo-EM. Published applications include the resolution of a self-splicing stage of Tetrahymena group I intron (2) and the structure of bacterial small non-coding RNA RsmZ (3). SimRNA-cry eases experimental RNA structure determination from low-resolution data and expands the scope of RNA structures that can be modeled.

References:

1. Boniecki et al., SimRNA: a coarse-grained method for RNA folding simulations and 3D structure prediction. Nucleic Acids Res. 2016 Apr 20;44(7):e63.

2. Luo et.al., Cryo-EM reveals dynamics of Tetrahymena group I intron self-splicing. Nat Catal 6, 298–309 (2023).

3. Jia, X. et al., Structural basis of sRNA RsmZ regulation of Pseudomonas aeruginosa virulence. Cell Res 33, 328–330 (2023).

**66**

## NACDDB: Nucleic Acid Circular Dichroism Database

**Andrea Cappannini**[1#], Kevin Mosca[2#], Sunandan Mukherjee[1], S. Naeim Moafinejad[1], Richard R. Sinden[3], Veronique Arluison[2,4], Janusz Bujnicki[1], Frank Wien[5]

[1] Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] Université Paris-Saclay, CEA, CNRS, Gif-sur-Yvette, France
[3] Department of Chemistry, Biology, and Health Sciences, South Dakota School of Mines and Technology, Rapid City, USA
[4] Université Paris Cité, Paris, France
[5] Synchrotron SOLEIL, L'Orme des Merisiers, Saint Aubin, Gif-sur-Yvette, France
[#] equal contribution

The Nucleic Acid Circular Dichroism Database (NACDDB) is a public repository that archives and freely distributes circular dichroism (CD) and synchrotron radiation CD (SRCD) spectral data about nucleic acids, and the associated experimental metadata, structural models, and links to literature. NACDDB we covered CD data for various nucleic acid molecules, including DNA, RNA, DNA/RNA hybrids, and various nucleic acid derivatives. Entries has been linked to primary sequence and experimental structural data, as well as to the literature. Additionally, for all entries, 3D structure models are provided. We meticulously validated and curated all entries to ensure the completeness, consistency, and quality of the data included. The NACDDB is open for submission of the CD data for nucleic acids.

NACDDB is available at: https://genesilico.pl/nacddb/.

Reference:

Andrea Cappannini et al., NACDDB: Nucleic Acid Circular Dichroism Database, Nucleic Acids Research, Volume 51, Issue D1, 6 January 2023, Pages D226–231, https://doi.org/10.1093/nar/gkac829.

This work has been awarded a Breakthrough Article status in Nucleic Acid Research.

67

## A curated dataset of RNA and RNP 3D structures with biologically relevant assemblies, with chemically modified residues and their unmodified counterparts

**Angana Ray**, Niloofar Shirvanizadeh, Iswarya Pandara Nayaka PJ, Sunandan Mukherjee, Masoud Amiri Farsani, Satyabrata Maiti, Janusz M. Bujnicki

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland

Naturally occurring RNAs encompass over 150 diverse modifications, each contributing significantly to RNA function. However, current research has yet to fully predict the impact of these modifications on RNA structure. Our objective is to devise a method for modeling the 3D structure of RNA molecules inclusive of modified residues and to understand the effect of modifications on RNA 3D structure. To this end, a critical step involves the detailed analysis of interactions within experimentally determined RNA 3D structures featuring modified residues. Consequently, we need to extract pertinent information from RCSB PDB database entries.

Molecular simulations pose specific requirements, particularly when comparing structures with and without naturally occurring modifications. These include complete atomic-level structures (no missing residues or atoms), avoidance of duplicate conformations, and biologically relevant multi-chain structures with the correct number and selection of chains. Unfortunately, these conditions are not consistently met by structures within the RCSB PDB database. Many structures feature missing atoms and residues, alternative conformations, and potentially unnatural chemical modifications, e.g., used to improve the structure determination process or to lock the structure in some specific functional state. Additionally, the structures in the asymmetric unit or in the biological assemblies do not always accurately represent the RNA molecules' assembly in the native environment.

To address these issues, we developed a computational pipeline tailored to process RNA structures from the RCSB PDB database. It either generates "repaired" RNA 3D coordinates suitable for molecular simulations or identifies structures that are challenging to repair, such as when residues are represented only by P atoms, thus complicating the reconstruction of an all-atom structure representation. Our pipeline also generates RNA structure variants by replacing modified residues (both natural and unnatural) with their corresponding unmodified counterparts (A, U, C, or G). The application of our pipeline yields two corresponding datasets of RNA and RNP structures differing solely in the presence or absence of modifications.

Leveraging these datasets, we analyzed the geometric interactions within RNAs containing modifications and compared them to their unmodified counterparts. Simultaneously, we computed a simple statistical potential that assesses the relative preference of residues to form canonical and non-canonical pairs. Our current work is now centered on expanding this analysis to include contacts mediated by modified residues.

**68**

## 3D structure determination of key regulatory RNAs at the 5' and 3’ untranslated regions of Flaviviruses

Tales Rocha de Moura, Nagendar Goud Badepally, **Elżbieta Purta**, Agata Bernat, Katarzyna Merdas, Janusz M. Bujnicki

Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology in Warsaw, Poland

The current global virus pandemic alerts us to the importance of better understanding the viruses' biology, including the untranslated RNA regions. Structural studies may pave the way not only to expand our knowledge of their function but also may lead to the development of novel RNA-targeted therapeutics. Flaviviruses are small, enveloped icosahedral viruses that possess a positive-strand RNA genome of 9–12 kb [1]. The viral RNA plays an active role in viral genome amplification, it provides RNA signals that act as promoters, enhancers, and/or silencers of the replication process [2]. RNA elements that modulate RNA replication were found at the 5' and 3' UTRs and within the viral coding sequence. The 5' end of the genome is described as a promoter for RNA synthesis, and it forms a large stem-loop structure [3, 4]. Our effort aims to solve the structures of these regulatory motifs, structured RNA elements in the 5’ and 3’ untranslated regions guiding viral replication, and RNA synthesis. For this purpose, we plan to follow an integrative approach using cryo-em, chemical mapping, and computational modeling to provide the possibility of determining the 3D structure of different flavivirus regulatory RNAs.

References:

1. Wang-Shick Ryu, Chapter 12 - Flaviviruses, Editor(s): Wang-Shick Ryu (2017) Molecular Virology of Human Pathogenic Viruses. Academic Press, Pages 165-175.

2. Gebhard, Leopoldo G.; Filomatori, Claudia V.; Gamarnik, Andrea V. (2011) Functional RNA Elements in the Dengue Virus Genome. Viruses 3, no. 9: 1739-1756.

3. Filomatori, C.V.; Lodeiro, M.F.; Alvarez, D.E.; Samsa, M.M.; Pietrasanta, L.; Gamarnik, A.V. (2006) A 5' RNA element promotes dengue virus RNA synthesis on a circular genome. Genes Dev., 20, 2238–2249.

4. Alvarez, D.E.; De Lella Ezcurra, A.L.; Fucito, S.; Gamarnik, A.V. (2005) Role of RNA structures present at the 3'UTR of dengue virus on translation, RNA synthesis, and viral replication. Virology, 339, 200–212.

**69**

## Development of methodology for quantitative analysis of tRNA modifications by nanopore RNA sequencing

Paula Martin-Arroyo[1], Katarzyna Piętka[1], Natalia Stróżyńska[1], Grażyna Leszczyńska[2], Robert Nowak[3], **Malgorzata Adamczyk**[1]

[1] Laboratory of Systems and Synthetic Biology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland
[2] Institute of Organic Chemistry, Faculty of Chemistry, Łódź University of Technology, Poland
[3] Institute of Computer Science, Faculty of Electronics and Information Technology, Warsaw University of Technology, Warsaw, Poland

Nanopore sequencing has been proven an efficient and reliable method for direct RNA sequencing. The method allows for evaluation of chemical modification present on mRNA. tRNA modifications are typically identified and quantified with high accuracy using liquid chromatography coupled to mass spectrometry (LC–MS) methodologies or NGS-based methods, which despite recent improvements suffer from technical pitfalls (Lucas et al. 2023). New efficient tRNA sequencing methods are still needed.

We have developed a new methodology called Nano-MODe-tRNAseq, a nanopore-based approach to sequence native tRNA molecules that offers quantitative estimation of tRNAs abundance and identification of ACL loop modifications. We designed synthetic, modified tRNAs oligonucleotides for full tRNAs assembling, and use them as internal standards in Nano-MODe-tRNAseq to enhance processing efficiency of raw nanopore current intensity signals obtained for natural tRNAs. An artificial neuronal network external model has been trained and will be utilised to identify specific signals containing elements characteristic to modifications in different tRNAs.

Research was funded by (POB Biotechnology and Biomedical Engineering) of Warsaw University of Technology within the Excellence Initiative: Research University (IDUB) programme. Project BIOTECHMED-3 Advanced no. 504/04496/1020/45.010421 founded to MA.

**70**

## HuR-targeted Eltrombopag and its implications on autophagy regulation

**Sivakumar Krishnamoorthy**[1#], Nathalie Idlin[1#], Natalia Stec[1], Christos Spanos[2], Nila Roy Choudhury[2], Chen Zhuo[3], Juri Rappsilber[3], Gracjan Michlewski[1,2]

[1] International Institute of Molecular and Cell Biology in Warsaw, Poland
[2] University of Edinburgh, UK
[3] Technische Universität Berlin, Germany
[#] equal contribution

Human antigen R (HuR/ELAVL1) is a ubiquitously expressed protein that belongs to Hu family of RBPs. Dysregulation of HuR has been implicated in various pathological conditions, including cancer, neurodegenerative disorders, inflammatory diseases, and viral infections. It plays a substantial role in post-transcriptional gene regulation through binding to the target mRNA and influence its efficiency in translation and stabilization. Inhibition of HuR can increase apoptosis and anti-inflammatory response in CLL (chronic lymphocytic leukaemia) cells, making them more sensitive to chemotherapy and other treatments. Even though few small molecules have been found that can block the function of HuR, they often lack a comprehensive analysis.

Eltrombopag is a thrombopoietin receptor agonist, a safe and effective orally administered medication to treat chronic immune thrombocytopenic purpura and chemotherapy-induced thrombocytopenia. Crucially, Eltrombopag has been shown to target HuR and elicit anti-angiogenic effects in breast cancer cells. Our study aims to elucidate the effects of an Eltrombopag on HuR and to uncover the complex molecular interactions between HuR and its downstream targets within the autophagy pathway. To achieve this, we performed quantitative, high-throughput analyses of mRNA and protein levels in the absence and presence of HuR as well as compared them to the treatment with Eltrombopag. The results from RNAseq analysis revealed global changes in gene expression, identifying potential regulatory mechanisms associated with autophagy. Furthermore, results of mass spectrometry provided insights into proteomic changes under same condition, shedding light on dynamic cellular responses. Finally, analysis of autophagy pathway showed significant changes exerted by HuR and Eltrombopag. In conclusion, these insights may hold promise for novel molecular events controlled by HuR and a potential for Eltrombopag to be used as effective anti-HuR medicine.

# Sponsors

## Sponsors of the Best Posters and Talks Awards

## Platinum Sponsor

## Silver Sponsors

LEXOGEN
The RNA Experts

Zakład Genetyki Sądowej
Authorized distributor in Poland

## RiboCop – Select and Deplete.

### Sequence what matters most with RiboCop rRNA Depletion.

**NEW!** Unlock yeast transcriptomics with RiboCop rRNA Depletion for Yeast.

Total RNA is comprised of large amounts of ribosomal RNA (rRNA) which can make up between ~80 – 98 % of all RNA molecules in a sample. Ribosomal RNA depletion removes these undesired transcripts to access transcripts of interest. Ultimately, rRNA depletion enables detection of all types of non-ribosomal RNAs, including coding mRNAs and non-coding transcripts such as snoRNAs, snRNAs, and lncRNAs. RiboCop rRNA Depletion Kits afford an undisturbed view of an organism's complete transcriptome and are available for various species, including human, mouse, rat, bacteria, and yeast.


**Preserve your RNA and process challenging samples with ease.**

Enzyme-free rRNA depletion preserves full-length RNA and is ideally suited for processing degraded and FFPE samples.

**Maximize your unique sequencing reads without introducing depletion bias.**

Sophisticated probe design allows efficient removal of undesired transcripts while maintaining unbiased transcriptome profiles.

**Easily scale up your experiments with RiboCop's automation-friendly workflow.**

RiboCop's convenient, magnetic bead-based workflow is compatible with automation on liquid handlers.

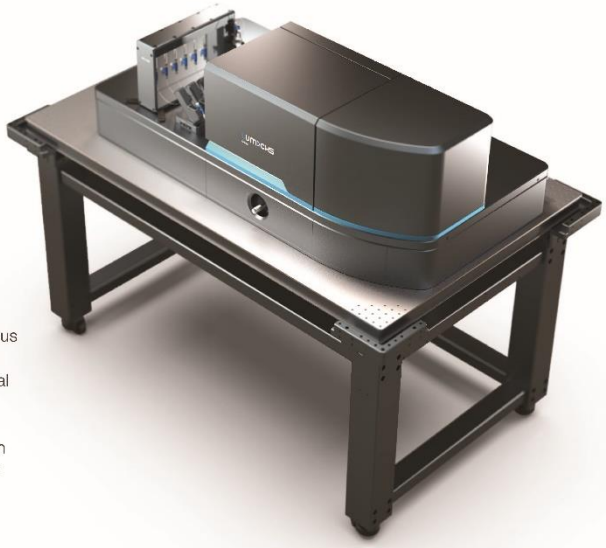
**Unlock the full potential of low input RNA.**

RiboCop supports the widest input range (1 ng – 1000 ng) with excellent performance for low input down to 1 ng total RNA.

For more information, visit our website lexogen.com or contact with our local distributor at orders@dna-zgs.pl

# C-Trap®

## Best-in-class **dynamic single-molecule microscopy**



Scan to download our brochures

The C-Trap® is the world's first instrument that allows simultaneous manipulation and visualization of single-molecule interactions in real time. It combines high resolution optical tweezers, fluorescence and label-free microscopy, and an advanced microfluidics system in a truly integrated and correlated solution.

## Single-molecule investigations of dynamic biological processes: from structure to function


- Track individual DNA-binding proteins in molecularly crowded environments and assess their dynamic properties under biological conditions.

**Read more**

Kaczmarczyk et al. **Nature Comm.** (2022)
Anand et al. **Nature** (2022)

- Introduce multiple fluorescently-labeled proteins and follow their interactions with a DNA or RNA molecule to study their activity.

Nguyen et al. **Nature Comm.** (2022)
Wasserman et al. **Cell** (2019)

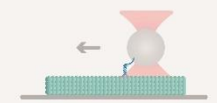

- Measure short-lived, long-lived, and rare conformational changes of single proteins, DNA or RNA molecules in real time and under different biological conditions.

**Read more**

Naqvi et al. **Science Advances** (2022)
Zimmer et al. **Nature Comm.** (2021)

- Combine optical tweezer measurements with FRET to correlate the mechanical properties of the protein to local structural changes.

Wruck et al. **Comm. Biology** (2021)
Avellaneda et al. **Nature** (2020)

- Assess highly dynamic processes of cytoskeletal remodeling and motor-protein transportation that are essential for molecular and cellular functions.

**Read more**

Siahaan et al. **Nature Chem. Bio.** (2022)
Schepers et al. **PNAS** (2021)

- Image filaments, motor and other proteins at the single-molecule level with high sensitivity and at video rate.

Lam et al. **Science Advances** (2021)
Schaedel et al. **Nature Comm.** (2021)




- Investigate changes to the phase of matter of protein droplets to understand condensate-associated cell functions and the development of pathological structures.

**Read more**

Alshareedah et al. **Nature Comm.** (2021)
Jaworth et al. **Science** (2020)


- Study receptor accumulation, filopodia formation and dynamics, localization, fusion and aggregation of cell components.

**Read more**

Evers et al. **iScience** (2022)
Vasse et al. **Small Methods** (2021)

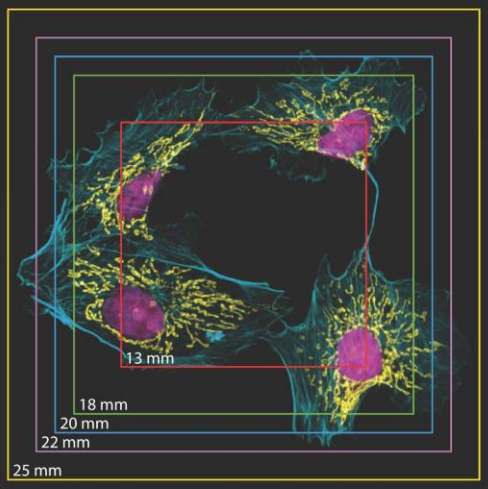
LUMICKS SPEKTROMETRIA

For more information reach out to Spektrometria, our local partner in Poland.
biuro@spektrometria.com.pl | spektrometria.com.pl

PRECOPTIC

Nikon

# Field of View



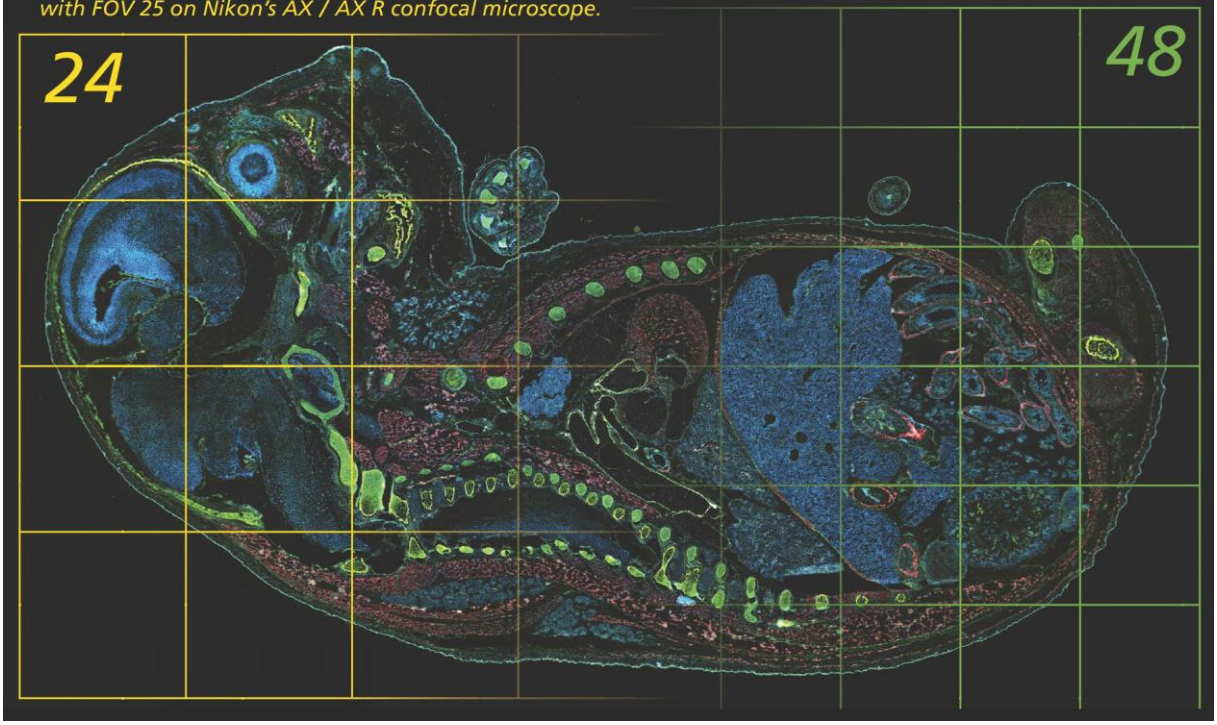

*Actin (cyan), mitochondria (yellow), and nuclei (magenta).*

## *Tiled acquisition*

When the entire region of interest cannot be captured in a single image field, overlapping "tiled" images can be acquired and merged into a single file. The FOV determines the number of tiled images needed to capture the entire specimen. Decreasing the number of locations imaged can significantly shorten the overall acquisition time.

*Entire region requires a total of about 24 frames with FOV 25 on Nikon's AX / AX R confocal microscope.*

*Entire region requires total of about 48 frames with conventional FOV 18.*

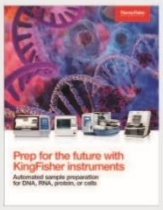


# RNA and beyond: Prep and analysis pocket library

## Support materials for automated sample preparation and downstream RNA analysis

## Flyers, brochures, and guides:

Thermo Scientific™ KingFisher™ instruments brochure




Invitrogen™ DynaGreen™ magnetic beads brochure


Nucleic acid kits by application and cell types flyer


DNA reference guide


RNA reference guide


Gene expression microarrays and assays brochure


## Videos and 3D tour:

Dynabeads magnetic beads: Cell isolation and activation video

KingFisher Apex Purification System video


KingFisher Apex Purification System 3D product tour

# Participants

| Name | Surname | Affiliation |
|---|---|---|
| **Małgorzata** | Adamczyk | Warsaw University of Technology |
| **Kamila** | Affek | International Institute of Molecular and Cell Biology in Warsaw |
| **Bharti** | Aggarwal | Adam Mickiewicz University, Poznań |
| **Matisa** | Alla | Institute of Human Genetics, Polish Academy of Sciences |
| **Angelika** | Andrzejewska-Romanowska | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Alicja** | Armatowska | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Nagendar Goud** | Badepally | International Institute of Molecular and Cell Biology in Warsaw |
| **Mateusz** | Bajczyk | Adam Mickiewicz University, Poznań |
| **Katarzyna** | Bandyra | University of Warsaw |
| **Emilia** | Baranowska | Celon Pharma S.A. |
| **Anna** | Barczak-Brzyżek | Warsaw University of Life Sciences |
| **Maciej** | Basczok | Adam Mickiewicz University, Poznań |
| **Anna** | Baud | Adam Mickiewicz University, Poznań |
| **Lakshmi** | Bellary | Institute of Human Genetics, Polish Academy of Sciences |
| **Gabriela** | Betlej | University of Rzeszow |
| **Urszula** | Białek-Wyrzykowska | International Institute of Molecular and Cell Biology in Warsaw |
| **Dominika** | Bloniarz | University of Rzeszow |
| **Leszek** | Błaszczyk | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Magdalena** | Boguta | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Agnieszka** | Bolembach | International Institute of Molecular and Cell Biology in Warsaw |
| **Michał** | Brouze | International Institute of Molecular and Cell Biology in Warsaw |
| **Aleksandra** | Brouze | International Institute of Molecular and Cell Biology in Warsaw |
| **Klaudia** | Bugara | Medical University of Silesia in Katowice |
| **Janusz** | Bujnicki | International Institute of Molecular and Cell Biology in Warsaw |
| **Kaspar** | Burger | University Hospital Wurzburg & University of Wurzburg |
| **Andrea** | Cappannini | International Institute of Molecular and Cell Biology in Warsaw |
| **Alfredo** | Castello | MRC-University of Glasgow Centre for Virus Research |
| **Maja** | Cieplak-Rotowska | IMol, Polish Academy of Sciences |
| **Maciej** | Cieśla | IMol, Polish Academy of Sciences |
| **Małgorzata** | Cieśla | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Agata** | Ciolak | Institute of Bioorganic Chemistry, Polish Academy of Sciences |

| | | |
|---|---|---|
| **Agnieszka** | Czarnocka-Cieciura | International Institute of Molecular and Cell Biology in Warsaw |
| **Rossella** | Debernardis | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |
| **Oleg** | Dmytrenko | Helmholtz Institute for RNA-based Infection Research |
| **Grazyna** | Dobrowolska | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Joanna** | Dodzian | International Institute of Molecular and Cell Biology in Warsaw |
| **Jakub** | Dolata | Adam Mickiewicz University, Poznań |
| **Martyna** | Plens-Gałąska | Adam Mickiewicz University, Poznań |
| **Karolina** | Drążkowska | University of Warsaw |
| **Eliana** | Dukhno | IMol, Polish Academy of Sciences |
| **Magdalena** | Dziembowska | University of Warsaw |
| **Andrzej** | Dziembowski | International Institute of Molecular and Cell Biology in Warsaw |
| **Agnieszka** | Faliszewska | International Institute of Molecular and Cell Biology in Warsaw |
| **Malak** | Farhat | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Katarzyna** | Fiedorowicz | International Institute of Molecular and Cell Biology in Warsaw |
| **Witold** | Filipowicz | Friedrich Miescher Institute for Biomedical Research |
| **Moh Egy Rahman** | Firdaus | IMol, Polish Academy of Sciences |
| **Agnieszka** | Fiszer | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Krzysztof** | Flis | Celon Pharma S.A. |
| **Daniel** | Gackowski | Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz |
| **Kamila** | Gajdek | International Institute of Molecular and Cell Biology in Warsaw |
| **Marta** | Gapinska | University of Warsaw |
| **Kishor** | Gawade | Adam Mickiewicz University, Poznań |
| **Karol** | Gawalski | Medical University of Warsaw |
| **Iga** | Gawrońska | University of Opole, Institute of Biology |
| **Maciej** | Gawroński | Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz |
| **Michał** | Gdula | Adam Mickiewicz University, Poznań |
| **Jingping** | Geng | University of Warsaw |
| **Piotr** | Gerlach | IMol, Polish Academy of Sciences |
| **Olga** | Gewartowska | International Institute of Molecular and Cell Biology in Warsaw |
| **Sebastian** | Glatt | Jagiellonian University, Malopolska Centre of Biotechnology |
| **Paulina** | Glazinska | Nicolaus Copernicus University in Toruń |
| **Anna** | Golisz-Mocydlarz | University of Warsaw |
| **Jakub** | Gołąb | Medical University of Warsaw |
| **Kinga** | Gołębiewska | Warsaw University of Life Sciences |
| **Daria** | Goś | International Institute of Molecular and Cell Biology in Warsaw |

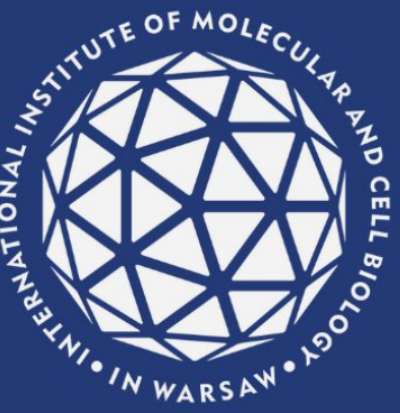
| | | |
|---|---|---|
| **Agnieszka** | Gozdek | University of Warsaw |
| **Maria** | Górna | University of Warsaw |
| **Max** | Graf | Research Institute of Molecular Pathology |
| **Renata** | Grzela | University of Warsaw |
| **Maria** | Grześ | Mossakowski Medical Research Institute, Polish Academy of Sciences |
| **Ewa Anna** | Grzybowska | Maria Skoldowska-Curie National Research Institute of Oncology |
| **Rajani Kanth** | Gudipati | Adam Mickiewicz University, Poznań |
| **Costanza** | Guidi | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |
| **Natalia** | Gumińska | International Institute of Molecular and Cell Biology in Warsaw |
| **Dilek Cansu** | Gurer | Izmir Institute of Technology |
| **Maria M** | Guzewska | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |
| **Veena** | Halale Manjunath | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Alexander** | Hammermeister | Jagiellonian University, Malopolska Centre of Biotechnology |
| | Ishani | IMol, Polish Academy of Sciences |
| **Olga** | Iwańska | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Ewa** | Jack-Górska | International Institute of Molecular and Cell Biology in Warsaw |
| **Maria** | Jagielska | University of Warsaw |
| **Akanksha** | Jaiswar | Mossakowski Medical Research Institute, Polish Academy of Sciences |
| **Marzena** | Jankowska-Anyszka | University of Warsaw |
| **Artur** | Jarmołowski | Adam Mickiewicz University, Poznań |
| **Jadwiga** | Jaruzelska | Institute of Human Genetics, Polish Academy of Sciences |
| **Magdalena** | Jazurek-Ciesiołka | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Jacek** | Jemielity | University of Warsaw |
| **Monika** | Jóźwiak | Adam Mickiewicz University, Poznań |
| **Monika** | Kaczmarek | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |
| **Ewelina** | Kałużna | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Kinga** | Kamieniarz-Gdula | Adam Mickiewicz University, Poznań |
| **Rafał** | Kampa | Medical University of Warsaw |
| **Karolina** | Kasztelan | International Institute of Molecular and Cell Biology in Warsaw |
| **Adrian** | Kasztelan | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Ashwini** | Khaladkar | IMol, Polish Academy of Sciences |
| **Elżbieta** | Kierzek | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Agnieszka** | Kiliszek | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Beata** | Kliszcz | Institute of Physics, Polish Academy of Sciences |
| **Marta** | Koblowska | University of Warsaw |

| | | |
|---|---|---|
| **Tim** | Kolberg | Leipzig University |
| **Adam** | Kolondra | Celon Pharma S.A. |
| **Magda** | Konarska | IMol, Polish Academy of Sciences |
| **Paweł** | Konieczny | Jagiellonian University, Medical College |
| **Magda** | Kopczyńska | Adam Mickiewicz University, Poznań |
| **Michał** | Koper | University of Warsaw |
| **Helena** | Kossowska | University of Warsaw |
| **Mariia** | Kovalenko | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Mateusz** | Kozarski | Celon Pharma S.A. |
| **Emilia** | Kozłowska | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Martyna** | Koźbiał | University of Warsaw |
| **Milena** | Krajewska | University of Warsaw |
| **Szymon** | Krasnodębski | University of Warsaw |
| **Paweł** | Krawczyk | International Institute of Molecular and Cell Biology in Warsaw |
| **Sivakumar** | Krishnamoorthy | International Institute of Molecular and Cell Biology in Warsaw |
| **Bart** | Krist | Adam Mickiewicz University, Poznań |
| **Joanna** | Krupka | University of Cambridge |
| **Wioletta** | Krysa | Medical University of Warsaw |
| **Michał** | Krzysztoń | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Dorota** | Kubacka | Celon Pharma S.A. |
| **Joanna** | Kufel | University of Warsaw |
| **Julia** | Kurzawska | Adam Mickiewicz University, Poznań |
| **Monika** | Kusio-Kobiałka | International Institute of Molecular and Cell Biology in Warsaw |
| **Joanna** | Kwiatkowska | Adam Mickiewicz University, Poznań |
| **Monika** | Kwiatkowska | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Przemysława** | Latoch | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Yaroslav** | Lavrynchuk | University of Łódź |
| **Fabian** | Leśniewski | Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz |
| **Dorota** | Libiszowska | International Institute of Molecular and Cell Biology in Warsaw |
| **Lidia** | Lipinska-Zubrycka | University of Warsaw |
| **Vladyslava** | Liudkovska | IMol, Polish Academy of Sciences |
| **Karolina** | Łabędzka-Dmoch | University of Warsaw |
| **Aleksandra** | Łopusińska | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Aneta** | Łyś | Adam Mickiewicz University, Poznań |
| **Gabriela** | Machaj | Jagiellonian University |
| **Łukasz** | Majewski | International Institute of Molecular and Cell Biology in Warsaw |
| **Michał** | Malewicz | Łukasiewicz Research Network - PORT Polish Center for Technology Development |

| | | |
|---|---|---|
| **Ewelina** | Małecka | International Institute of Molecular and Cell Biology in Warsaw |
| **Rafał** | Mańka | University of Opole |
| Magda | Masłoń | Adam Mickiewicz University, Poznań |
| **Dominika** | Maziarz | University of Opole |
| **Michal** | Mazur | International Institute of Molecular and Cell Biology in Warsaw |
| **Rahul** | Mehta | Jagiellonian University, Malopolska Centre of Biotechnology |
| **Jadwiga** | Meissner | IMol, Polish Academy of Sciences |
| **Katarzyna** | Merdas | International Institute of Molecular and Cell Biology in Warsaw |
| **Marta** | Miączyńska | International Institute of Molecular and Cell Biology in Warsaw |
| **Gracjan** | Michlewski | International Institute of Molecular and Cell Biology in Warsaw |
| **Jan** | Mikołajczyk | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Mario** | Mörl | Leipzig University |
| **Sunandan** | Mukherjee | International Institute of Molecular and Cell Biology in Warsaw |
| **Nikola** | Musiała | Adam Mickiewicz University, Poznań |
| **Katarzyna** | Niedźwiecka | University of Warsaw |
| **Martyna** | Nowacka | Molecure S.A. |
| **Elżbieta** | Nowak | International Institute of Molecular and Cell Biology in Warsaw |
| **Dominika** | Nowis | Medical University of Warsaw |
| **Marcin** | Nowotny | International Institute of Molecular and Cell Biology in Warsaw |
| **Joanna** | Nynca | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |
| **Marcin** | Ogonowski | International Institute of Molecular and Cell Biology in Warsaw |
| **Mikołaj** | Olejniczak | Adam Mickiewicz University, Poznań |
| **Ewelina Patrycja** | Owczarek | International Institute of Molecular and Cell Biology in Warsaw |
| **Katarzyna** | Pachulska-Wieczorek | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Anna** | Pacwa | Medical University of Silesia in Katowice |
| **Lori** | Passmore | MRC Laboratory of Molecular Biology, Cambridge |
| **Małgorzata** | Perycz | Institute of Computer Science, Polish Academy of Science |
| **Agnieszka** | Piasecka | Adam Mickiewicz University, Poznań |
| **Anna** | Piaszyk-Borychowska | Adam Mickiewicz University, Poznań |
| **Jakub** | Piątkowski | University of Warsaw |
| **Jan** | Piechna | International Institute of Molecular and Cell Biology in Warsaw |
| **Karolina** | Piecyk | University of Warsaw |
| **Iwona** | Pilecka | International Institute of Molecular and Cell Biology in Warsaw |

| | | |
|---|---|---|
| **Przemysława** | Płociński | University of Łódź |
| **Agnieszka** | Potęga | International Institute of Molecular and Cell Biology in Warsaw |
| **Monika** | Puczyńska | International Institute of Molecular and Cell Biology in Warsaw |
| **Elżbieta** | Purta | International Institute of Molecular and Cell Biology in Warsaw |
| **Dorota** | Raczyńska | Adam Mickiewicz University, Poznań |
| **Eliza** | Ratkowska | International Institute of Molecular and Cell Biology in Warsaw |
| **Agana** | Ray | International Institute of Molecular and Cell Biology in Warsaw |
| **Andrea** | Rentmeister | University of Münster |
| **Daria** | Riabov | Research Institute of Molecular Pathology |
| **Michaela** | Ristová | University of Edinburgh |
| **Tales** | Rocha de Moura | International Institute of Molecular and Cell Biology in Warsaw |
| **Izabela** | Rudzińska | Celon Pharma S.A. |
| **Sebastian** | Sacharowski | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Upasana** | Saha | Adam Mickiewicz University, Poznań |
| **Klaudia** | Samorowska | Adam Mickiewicz University, Poznań |
| **Bertrand** | Seraphin | Institute of Ganetics, Molecular and Callular Biology |
| **Małgorzata** | Sierant | Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences |
| **Paweł** | Sikorski | University of Warsaw |
| **Jakub** | Skaruz | International Institute of Molecular and Cell Biology in Warsaw |
| **Jakub** | Skibiński | University of Łódź |
| **Krzysztof** | Skowronek | International Institute of Molecular and Cell Biology in Warsaw |
| **Klaudia** | Skrzypek | Jagiellonian University, Medical College |
| **Krzysztof** | Sobczak | Adam Mickiewicz University, Poznań |
| **Anna** | Stankiewicz-Drogoń | University of Warsaw |
| **Marta** | Starczak | Nicolaus Copernicus University in Toru, Collegium Medicum in Bydgoszcz |
| **Agata** | Starosta | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Klaudia** | Staszak | Adam Mickiewicz University, Poznań |
| **Filip** | Stefaniak | International Institute of Molecular and Cell Biology in Warsaw |
| **Ewa** | Stein | Adam Mickiewicz University, Poznań |
| **Agata** | Stępień | Adam Mickiewicz University, Poznań |
| **Ewa** | Stępniak-Konieczna | Adam Mickiewicz University, Poznań |
| **Ramasamy** | Subbiah | Madurai Kamaraj University |
| **Aleksandra** | Sulkowska | University of Potsdam |

| | | |
|---|---|---|
| **Szymon** | Swiezewski | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Michał** | Szcześniak | Adam Mickiewicz University, Poznań |
| **Łukasz** | Szewc | Adam Mickiewicz University, Poznań |
| **Marta** | Sztachera | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Agata** | Szulim | International Institute of Molecular and Cell Biology in Warsaw |
| **Jacek** | Szymański | International Institute of Molecular and Cell Biology in Warsaw |
| **Patrycja** | Świergiel | Adam Mickiewicz University, Poznań |
| **Bartosz** | Tarkowski | International Institute of Molecular and Cell Biology in Warsaw |
| **Ivan** | Trus | International Institute of Molecular and Cell Biology in Warsaw |
| **Tomasz** | Turowski | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Katarzyna** | Tutak | Adam Mickiewicz University, Poznań |
| **Ewa** | Tykwińska | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Barbara** | Uszczyńska-Ratajczak | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Kevork** | Wakimian | IMol, Polish Academy of Sciences |
| **Marta** | Walczak | Jagiellonian University, Malopolska Centre of Biotechnology |
| **Dawid** | Walerych | Mossakowski Medical Research Institute, Polish Academy of Sciences |
| **Elzbieta** | Wanowska | Adam Mickiewicz University, Poznań |
| **Weronika** | Wendlandt-Stanek | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Tomasz** | Węgierski | International Institute of Molecular and Cell Biology in Warsaw |
| **Agnieszka** | Wilamowska | International Institute of Molecular and Cell Biology in Warsaw |
| **Magdalena** | Wołczyk | International Institute of Molecular and Cell Biology in Warsaw |
| **Magdalena** | Wrona | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Ruslan** | Yatusevich | Institute of Biochemistry and Biophysics, Polish Academy of Sciences |
| **Guillem** | Ylla | Jagiellonian University |
| **Monika** | Zakrzewska-Płaczek | University of Warsaw |
| **Anna** | Zawada | Cardinal Stefan Wyszynski University in Warsaw |
| **Małgorzata** | Zawadzka | Institute of Bioorganic Chemistry, Polish Academy of Sciences |
| **Agnieszka** | Ziemka | International Institute of Molecular and Cell Biology in Warsaw |
| **Joanna** | Zwolenkiewicz | Adam Mickiewicz University, Poznań |
| **Beata** | Zygmunt | Celon Pharma S.A. |
| **Daniel** | Żarski | Institute of Animal Reproduction and Food Research, Polish Academy of Sciences |




**International Institute of Molecular and Cell Biology in Warsaw**
4 Ks. Trojdena Street, 02-109 Warsaw, Poland
www.iimcb.gov.pl