

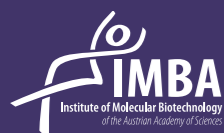
19. MICROSYMPOSIUM ON RNA BIOLOGY



PROGRAMME

19th Microsymposium on RNA Biology

Vienna BioCenter, May 7th - 9th 2025



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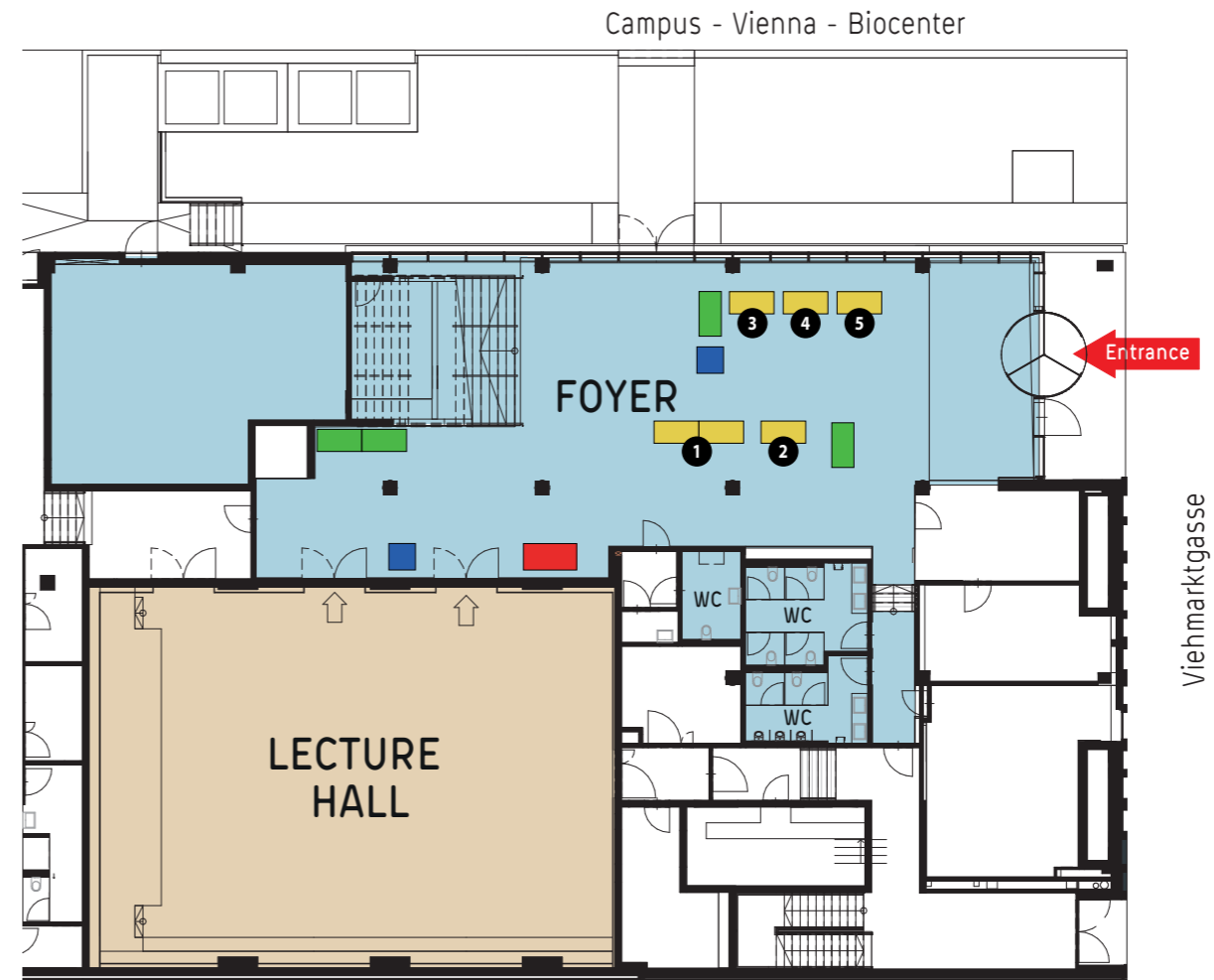
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19th MICROSYMPOSIUM on RNA Biology

DAY 1 - Wednesday, May 07

10:00 **Registration**

11:30 **Welcome & introduction**

11:45 **Luisa Cochella** (Johns Hopkins School of Medicine)
An essential and ancient miRNA family controls cell interaction pathways in *C. elegans*

12:15 **Gesa Hoffmann** (Max-Planck-Institute of Molecular Plant Physiology)
AGO5 restricts plant virus vertical transmission in gametes

12:35 **Massimiliano Clamer** (IMMAGINA)
Uncovering stress-induced responses in the tRNAome of actively translating ribosomes

12:45 *lunch break*

13:45 **Peter Brodersen** (University of Copenhagen)
A proxy RNAi pathway for sensing pathogenic cytoplasmic double-stranded RNA in plants

14:15 **Carrie Bernecky** (Institute of Science and Technology Austria)
A phosphorylated form of MDA5 influences its ATP-responsive RNA binding activity

14:45 *coffee break*

15:15 **Sandra Duhaucourt** (Université Paris Cité / CNRS)
Control of transposable elements by small RNA guided Polycomb in the unicellular eukaryote *Paramecium*

15:45 **Fabian Poetz** (Friedrich Miescher Institute for Biomedical Research)
Comprehensive interrogation of the mouse TFome reveals an unexpected connection between Ccr4-Not and snRNA biogenesis

16:05 **William Garland** (Aarhus University)
The Integrator complex regulates RNA Polymerase III transcription events

16:25 *coffee break*

16:55 **PhD workshop pt.1**

Júlia Portell Montserrat (IMBA/IMP)
Target engagement by Piwi-piRNA complexes promotes the stepwise assembly of a nuclear silencing machinery

Ece Turkmenoglu (Max Delbrueck Center)
Assessing Roles of Upstream Open Reading Frames in Early Zebrafish Development through in vivo CRISPR screening

Lizaveta Pshanichnaya (IMB Mainz)
A role for the intrinsically disordered N-terminal region of WAGO-3 in small RNA selection

17:55 *Poster session I*

19:25 *symposium dinner at venue*

DAY 2 – Thursday, May 08

09:00 **Hauke Hillen** (Max Planck Institute for Multidisciplinary Sciences) - **EMBO YIP Lecture**
Biogenesis of organellar and viral RNA

09:30 **Dagmar Zigackova** (Yale University)
The “all-or-none” pre-mRNA processing of globin transcripts

09:50 *coffee break*

10:20 **PhD workshop pt.2**

Glenn Li (MIT/Whitehead Institute/HHMI)
Stabilization of excised introns in yeast

Thomas Kapral (Max Perutz Labs & University of Vienna)
Large-scale organization of RNA-protein interaction networks reflects structure of the genetic code

Haejeong Lee (Yale University)
Deciphering the code: codons, codon-codon interactions, amino acids, and peptide bonds regulate mRNA

stability

11:20 *coffee break*

11:50 **Tineke Lenstra** (Netherlands Cancer Institute) - **EMBO YIP Lecture**
Understanding transcription: one molecule at a time

12:20 **Eleonora Perego** (University of Lausanne)
Pioneer transcription factor Nanog stabilizes DNA-TF clusters to facilitate transcription in early zebrafish embryos.

12:40 **Mounia Lagha** (Institut de Génétique Moléculaire) - **EMBO YIP Lecture**
Translation dynamics during zygotic genome activation

13:10 *group picture & lunch*

14:10 **Adam Cawte** (University of Oxford)
Visualisation of nuclear ncRNAs in living cells using Nuclear Background-reduced RNA-Imaging with Tamoxifen and ERT2 (Nuclear-BRITE)

14:30 **Stirling Churchman** (Harvard Medical School)
Dynamics of Gene Regulation, from the Nucleus to Mitochondria

15:00 *coffee break*

15:30 **PhD workshop pt.3**

Aileen-Diane Bamford (ETH Zurich)
Live single-transcript imaging reveals global shift in particle dynamics during neuronal development

Moritz Leitner (Max Perutz Labs & University of Vienna)
Ashwin localizes the tRNA ligase complex to the nucleus for pre-tRNA splicing

16:10 *Poster session II*

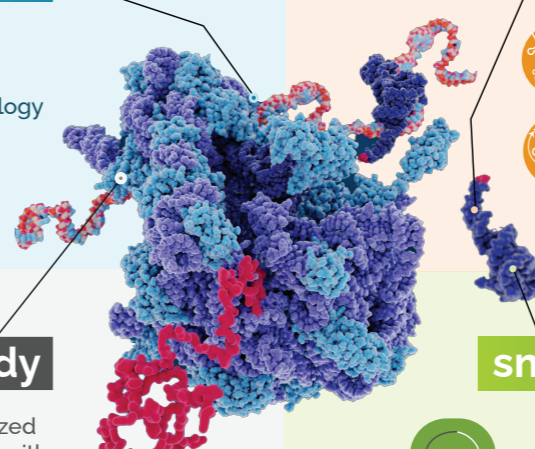
17:40 End of poster session - free evening for attendees

DAY 3 – Friday, May 09

09:00	Todd Blevins (Centre national de la recherche scientifique) Functional assembly and regulation of the RNA polymerase IV machinery in plants
09:30	Marianne Yoth (Aarhus University) Two phases of transposon regulation in Drosophila germline development
09:50	Douglas Black (University of California, Los Angeles) Splicing regulation at the U2 snRNP-branchpoint complex.
10:20	<i>coffee break</i>
10:50	Sebastian Glatt (University of Veterinary Medicine Vienna & Jagiellonian University Krakow) tRNAslational control of eukaryotic gene expression
11:20	Yong Xiong (Yale School of Medicine) High-Resolution Translation Landscapes in Human Cells
11:50	<i>coffee break</i>
12:20	Frederick Rehfeld (UT Southwestern Medical Center) A genome-wide screen reveals that oxidative stress regulates translation elongation to promote production of detoxifying selenoproteins
12:40	Rachel Green (Johns Hopkins School of Medicine) Concentration matters: Regulation of ribosome homeostasis during cellular stress
13:10	awards & closing
13:30	<i>farewell lunch</i>



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Session speakers day 1

An essential and ancient miRNA family controls cell interaction pathways in *C. elegans*

Luisa Cochella

Johns Hopkins School of Medicine

AGO5 restricts plant virus vertical transmission in gametes

Gesa Hoffmann, Marco Incarbone

Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany

Viruses are intracellular parasites that rely on transmission to new hosts to ensure their continued existence. Vertical transmission—the passage of viral infection from parent to progeny—is a key mechanism for virus persistence across generations and geographical regions. This mode of transmission is especially significant in plants, where viruses spread both locally and globally via pollen and seeds. However, vertical transmission in plants is often inefficient or entirely absent, suggesting the existence of potent transgenerational antiviral barriers which remain unknown (Bradamante et al., 2021; Hoffmann & Incarbone, 2024). In this study, we demonstrate that AGO5, an RNA interference factor uniquely expressed in stem cells and gametes, plays a crucial role in restricting the vertical transmission of Turnip yellow mosaic virus (TYMV) in *Arabidopsis thaliana*. Using reciprocal crosses, we show that AGO5 suppresses viral transmission through both male and female parents. Analysis of heterozygous ago5 knock-out mutants revealed that AGO5 restricts vertical transmission post-meiosis and pre-fertilization during host reproduction, emphasizing its gamete-specific antiviral activity while excluding involvement in the zygote or embryo. Moreover, production of virus-specific small RNAs in sperm cells significantly reduced pollen-mediated virus transmission. Similarly, sperm cell-specific expression of AGO5 in ago5 mutants restored antiviral activity, further validating the gamete-specific function of AGO5. Collectively, our findings unveil the first known antiviral mechanism restricting RNA virus transmission from parent to progeny through gametes, a significant advance that will increase our toolkit in the management of viral diseases.

Uncovering stress-induced responses in the tRNAome of actively translating ribosomes

Massimiliano Clamer

Immagina Biotechnology

Living organisms adapt to environmental changes by regulating gene expression at multiple levels, including protein synthesis. During translation, tRNA molecules deliver specific amino acids to the ribosome in a manner coordinated with the sequence encoded in the messenger RNA. However, the precise role of tRNAs, including their modifications, in modulating translation remains poorly understood due to lack of methods to map ribosome selected tRNAs. Traditional analyses of tRNA dynamics often focus on total cellular tRNAs, which can mask subtle, stress-induced changes. To address this challenge, we developed a method to isolate ribosome-associated tRNAs from actively translating ribosomes and applied direct nanopore sequencing to compare these functionally active tRNAs with the total cellular pool in immortalized human cells. This approach combines antibody- and tag-free pull-down of active ribosomes with multiplexed sequencing, enabling comprehensive and comparative measurements of tRNA abundance, charging, and modifications in both the total and ribosome-embedded RNA pools.

A proxy RNAi pathway for sensing pathogenic cytoplasmic double-stranded RNA in plants

Peter Brodersen

University of Copenhagen

Sensors of intracellular double-stranded RNA are central components of metazoan innate antiviral immunity, but such sensors have not been identified in plants. RNA interference (RNAi) constitutes a potent plant antiviral defense mechanism that relies on conversion of viral RNA into small interfering RNAs by two DICER-LIKE (DCL) ribonucleases, DCL4 and DCL2. I will present results that indicate that while DCL4 and DCL2 are both required for antiviral resistance, their roles are completely different. DCL4 is dedicated to RNAi in the classical sense. On the contrary, cytoplasmic dicing by DCL2 also triggers RNAi-independent defense gene expression via at least two intracellular nucleotide-binding domain/leucine-rich repeat (NLR) immune receptors. Deficiencies in both RNAi and intracellular dsRNA sensing obtained by DCL4/NLR inactivation abrogates basal resistance to a positive strand RNA virus. These results redefine the basis of plant antiviral immunity, and introduce autoimmunity as an explanation for DCL2-dependent growth arrest in dcl and RNA decay mutants in several plant species.

A phosphorylated form of MDA5 influences its ATP-responsive RNA binding activity

Carrie Bernecky

Institute of Science and Technology Austria

Human melanoma differentiation-associated protein 5 (MDA5) is a cytosolic sensor of double-stranded RNA that activates the innate immune response during viral infection. In addition to its protective role against pathogens, MDA5 has been associated with a variety of inflammatory and autoimmune disorders through the pathogenic recognition of cellular RNAs. Although many regulatory mechanisms have been discovered, how post-translational modifications control MDA5 activity is incompletely understood. Here, we report the identification of a phosphorylated form of MDA5 with an altered response to ATP. In this talk, I will present our biochemical and structural work towards uncovering how phosphorylation affects ATP-responsive RNA-binding specificity by a key innate immunity sensor.

Control of transposable elements by small RNA guided Polycomb in the unicellular eukaryote *Paramecium*

Sandra Duharcourt

Université Paris Cité / CNRS

Comprehensive interrogation of the mouse TFome reveals an unexpected connection between Ccr4-Not and snRNA biogenesis

Fabian Poetz (1), Filip Pekovic (2), Michaela Schwaiger (1), Daniel Hess (1), Jan Seebacher (1), Sebastien Smallwood (1), Eugene Valkov (2), Marc Buehler (1,3)

(1) Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland

(2) RNA Biology Laboratory, Center for Cancer Research, National Cancer Institute (NCI), Frederick, USA

(3) University of Basel

The evolutionarily conserved Ccr4-Not complex functions as a major mRNA deadenylase and acts as a central player in the regulation of post-transcriptional gene expression. Although Ccr4-Not subunits were first identified as transcriptional regulators in yeast genetic screens, there is currently no evidence of a nuclear function in higher eukaryotes. In an attempt to comprehensively profile the interactomes of all transcription factors (TFs) expressed in mouse embryonic stem cells by IP-MS, we have identified the two poorly characterized putative TFs Gbbp1 and its paralog Gbbp111 as novel interactors of Ccr4-Not. Structure predictions and in vitro interaction assays reveal a direct interaction between Gbbp1 and two distinct modules of Ccr4-Not. While our systematic IP-MS approach identified the interaction with Ccr4-Not, ChIP-Seq revealed a specific occupancy of Gbbp1 and Gbbp111 on actively Pol2-transcribed snRNA repeats. Moreover, ChIP-Seq signals for both TFs at snRNA repeats strongly correlate with ChIP-Seq signals for Cnot1, a scaffold component of the Ccr4-Not complex, and other catalytic and non-catalytic subunits, thus opening up the possibility that Ccr4-Not may be recruited to Pol2-transcribed snRNA repeats. Given that the binding of Gbbp1 and Gbbp111 to snRNA repeats relies on active transcription, we currently investigate whether Gbbp1 and its paralog recruit Ccr4-Not in a manner dependent on nascent RNA, and aim to understand the functional relevance of these interactions for snRNA biogenesis.

The Integrator complex regulates RNA Polymerase III transcription events

William Garland, Jérôme O. Rouvière, Lis Jakobsen, Kristoffer Pors Jakobsen, Guifen Wu, Søren Lykke-Andersen, Jens S. Andersen and Torben Heick Jensen

Aarhus University

The Integrator (INT) complex is well established as an attenuator of non-productive RNA Polymerase II (RNAPII) transcription events through its transcription termination activities. However, the function of Integrator with other RNA polymerases has not been explored. Here, we report that INT can also function to regulate RNAPIII transcription events. Using proximity labelling and interaction approaches in mouse ES cells, we show that INT and RNAPIII co-localise in vivo. This is corroborated by global ChIPseq profiles demonstrating that INT is enriched at RNAPIII loci. Upon rapid depletion of INTS1, we observe a general increase in RNAPIII levels at its target loci and downstream regions reminiscent of analogous observations with RNAPII. In line with this, we also observe an increase in unstable 3' extended RNAs generated from RNAPIII genes following INTS1 depletion. Taken together, we suggest that Integrator is not exclusive to RNAPII but can also function to regulate the activity of RNAPIII, thereby expanding the repertoire of its functional activities.

PhD workshop pt.1

Target engagement by Piwi-piRNA complexes promotes the stepwise assembly of a nuclear silencing machinery

Júlia Portell-Montserrat^{1,2,3}, Laszlo Tirian¹, Changwei Yu¹, Giacomo Silvestri¹, Ulrich Hohmann^{1,2}, Dominik Handler¹, Laura Fin², Clemens Plaschka², Julius Brennecke¹

¹ Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC); Dr. Bohr-Gasse 3, 1030 Vienna, Austria

² Institute of Molecular Pathology (IMP), Campus Vienna BioCenter, 1030 Vienna, Austria

³ Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Vienna, Austria

PIWI proteins—bound to piRNAs—protect animal genomes from the activity of transposable elements. They enforce transposon silencing through distinct mechanisms depending on their cellular localization: in the cytoplasm, they trigger target RNA cleavage and piRNA amplification, while in the nucleus, they promote heterochromatin formation to repress transcription. However, how PIWI-piRNA complexes selectively recruit downstream effectors to initiate these distinct silencing pathways remains unclear. Here, we focus on the *Drosophila* nuclear pathway to investigate the molecular mechanisms underlying PIWI-mediated silencing.

By combining structural predictions with biochemical and genetic analyses, we show that piRNA-guided target recognition leads to the formation of a nuclear silencing hub, which we term the Piwi* complex. This assembly comprises Piwi, the zinc-finger protein Asterix/Gtsf1, and the RNase H-like protein Maelstrom. The Piwi* complex acts as a central platform, engaging both the heterochromatin-inducing SFI-X complex and the RNA helicase Spindle-E via distinct interfaces. Strikingly, analogous PIWI* complexes also form in the cytoplasm with Aubergine and Argonaute-3, revealing a conserved mechanism for coupling target recognition to effector recruitment.

These findings uncover a unifying and evolutionarily conserved mechanism by which PIWI-piRNA complexes couple target RNA recognition with the recruitment of effector proteins, orchestrating distinct silencing programs in different cellular compartments.

Assessing Roles of Upstream Open Reading Frames in Early Zebrafish Development through in vivo CRISPR screening

Ece Türkmenoglu^{1, 2}, Scott A. Lacadie¹, Uwe Ohler^{1, 2, 3}

¹ Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association

² Department of Biology, Humboldt-Universität zu Berlin, Berlin, Germany

³ Department of Computer Science, Humboldt-Universität zu Berlin, Berlin, Germany

Translation is a tightly regulated step in gene expression. After transcription, mRNA levels can only partially explain protein abundance and variability in cells, indicating that the proteome is regulated at the level of translation. Translation initiation can be controlled through short sequence elements on mRNA which are present in the 5' leader sequence of the transcripts, named upstream open reading frames (uORF). uORFs have a start codon from which the ribosome can initiate translation before reaching the start codon of the downstream coding gene. Thus, uORFs can influence the translation of the main ORF and affect protein expression.

To investigate the role of uORFs in gene expression regulation during early development, we generated zebrafish ribosome profiling data across different embryonic stages. Our analysis identified and annotated over 1500 uORFs, including dozens that exhibit changes in translational activity. Additionally, we performed a conservation analysis by aligning nucleotide and amino acid sequences of uORFs to determine evolutionarily conserved uORFs. We observed genes with translationally dynamic and conserved uORFs that may play essential roles, particularly in key signaling pathways and tissue development.

To further explore the function of dynamic and conserved uORFs, we established an in vivo CRISPR screen in zebrafish embryos. Using base editing, we will target uORF start codons with guide RNAs, deliver CRISPR components into embryos, and assess resulting phenotypic and molecular changes.

By integrating ribosome profiling with in vivo CRISPR screening, this project aims to provide a deeper understanding of uORF function and translational regulation during vertebrate development.

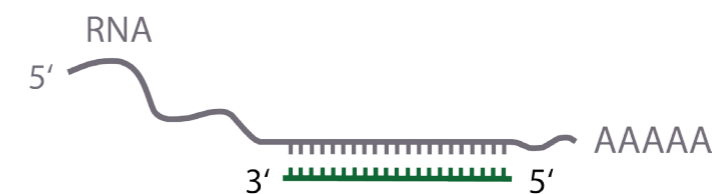
A role for the intrinsically disordered N-terminal region of WAGO-3 in small RNA selection

Lizaveta Pshanichnaya, Jan Schreier, Svenja Hellmann, René Ketting
IMB Mainz

Small RNA-mediated gene regulatory pathways have been identified across all domains of life. Key players of these pathways are Argonaute proteins that bind small RNAs and guide them to complementary target transcripts. *Caenorhabditis elegans* has a distinct class of Argonautes known as Worm-specific Argonaute proteins (WAGOs). Operating as secondary Argonautes, WAGOs acquire their small RNA through intricate processes involving 'primary' Argonaute proteins and small RNA amplification. As other proteins from this family, WAGOs consist of four major structural domains: PAZ, MID, PIWI, N-terminal domain. In addition, WAGOs have an N-terminal intrinsically disordered region (IDR). Previous crystallography studies have highlighted the significance of the PAZ and MID domains in RNA binding, while the PIWI domain is responsible for RNA cleavage. However, the role of N-terminal IDR remains unclear. Our study is focused on understanding the function of N-terminal IDR in WAGOs, using WAGO-3 as a model protein.

WAGO-3 is known to bind WAGO class 22G small RNA and to be important for paternal inheritance³. While wago-3 mutants do display defects, the absence of WAGO-3 does not induce a dramatic or acute fertility phenotype. Interestingly, our studies showed that WAGO-3 lacking the N-terminal IDR induces a strong, dominant and acute effect on germline formation, resulting in full sterility. Controlling WAGO-3 expression level using the auxin-inducible degradation system we were able to conduct RNA immunoprecipitation of WAGO-3 lacking its N-terminal IDR region. Results showed that IDR deletion led to the binding of small RNAs beyond the usual WAGO-interacting 22G RNAs, suggesting that the IDR controls WAGO-3 loading. Ongoing investigations support the idea that these non-specific 22G RNAs cause the acute phenotype. We currently aim to better understand the details of how the N-terminal IDR may control WAGO small RNA binding.

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Session speakers day 2

Biogenesis of organellar and viral RNA

Hauke Hillen

Max Planck Institute for Multidisciplinary Sciences

RNA biogenesis in eukaryotic cells is not confined to the nucleus, but also takes place in other parts of the cell. For example, mitochondria and chloroplasts contain organellar genomes that are expressed by specialized machineries, and many RNA viruses replicate and transcribe their genomes in the cytoplasm. However, the molecular mechanisms of these gene expression systems remain relatively poorly understood. In our group, we use a combination of structural biology and biochemical as well as cellular methods to study organellar and viral gene expression, with a particular focus on RNA biogenesis and maturation. In my talk, I will provide an overview of our research and highlight recent advances in our understanding of mitochondrial, chloroplast and viral RNA biogenesis.

The “all-or-none” pre-mRNA processing of globin transcripts

Dagmar Zigackova, Morgan Shine, Korinna Straube, Vandana Kumari, Manoj Pillai and Karla Neugebauer

Yale University, New Haven, CT, USA

The Neugebauer lab studies how RNA processing events are coordinated with each other and with transcription using long-read sequencing (LRS) of nascent RNA and mRNA. We have quantified pre-mRNA splicing and polyA site (PAS) cleavage, revealing co-transcriptional splicing kinetics in budding and fission yeasts and mouse cells. In the yeasts, a subpopulation of transcripts fully unspliced and uncleaved at PAS contrasted with the majority of efficiently processed nascent transcripts. We refer to this phenomenon as “all-or-none” pre-mRNA processing. In *S. pombe*, many of the “none” transcripts were co-transcriptionally degraded. Mammalian all-or-none pre-mRNA processing was observed in murine erythroleukemia (MEL) cells undergoing erythropoiesis. Surprisingly, both α - and β -globin genes were among the most prominent examples, with a 50:50 ratio of all processed vs. all unprocessed nascent transcripts. A mutation leading to more efficient splicing of β -globin transcripts also improved PAS cleavage. These data suggest that PAS cleavage is promoted by splicing and blocked in unspliced transcripts. It is surprising that, despite the need for a massive production of hemoglobin in erythroid cells, only half of the nascent globin transcripts are fully processed.

What is the fate and function of “none” globin transcripts? First, we tested if all-or-none pre-mRNA processing of globin takes place in the HUDEP2 cell line, an accepted model for human erythropoiesis. Indeed, human and mouse β -globin show the same 50:50 all-or-none behavior. To follow the fate of the unprocessed transcripts in HUDEP2 cells we performed LRS of nuclear and cytoplasmic polyA+ RNA. The data show that β -globin cytoplasmic transcripts do not contain introns suggesting that only fully processed RNAs are exported into cytoplasm. Interestingly, partially processed nuclear β -globin transcripts were found by LRS of total nuclear RNA. These transcripts are unspliced but cleaved at the canonical PAS, suggesting that unspliced nascent transcripts are delayed – not blocked – in their cleavage as compared to spliced transcripts. We adapted our LRS protocol to count the lengths of polyA-tails with single nt accuracy. Cleaved and unspliced β -globin transcripts possess polyA-tails ~100 nucleotides longer than those of cleaved and spliced transcripts. This trend was consistent across the whole transcriptome, indicating that spliced transcripts might undergo par-

tial deadenylation before export to the cytoplasm. We will further discuss whether these partially processed transcripts are subjected to degradation in human cells or whether they are post-transcriptionally spliced, thereby serving as a reservoir for mRNA for later stages of red blood cell development.

Understanding transcription: one molecule at a time

Tineke Lenstra

Netherlands Cancer Institute

Transcription in single cells is dynamic, resulting in heterogeneity in gene expression in a population. In our lab, we aim to understand the mechanisms that regulate transcription dynamics in single cells. We use a range of single-molecule imaging techniques to directly observe the stochastic behavior of regulatory factors and the process of transcription inside living cells. An important step in gene regulation is the binding of gene-specific transcription factors to the DNA. In this talk, I will focus on how transcription factors find their target loci in living cells, what regulates their DNA binding kinetics, and how transient DNA binding of transcription factors contribute to gene activation.

Pioneer transcription factor Nanog stabilizes DNA-TF clusters to facilitate transcription in early zebrafish embryos.

Eleonora Perego, Noémie M. Chabot, Ramya Purkanti, Shivali Dongre, Alessia Del Panta Ridolfi, Damian Dalle Nogare, Haruka Oda, Hiroshi Kimura, Florian Jug, Alma Dal Co, Nadine L. Vastenhouw

University of Lausanne (UNIL)

Transcription factor (TF) clustering enhances gene activation by promoting interactions between TFs, chromatin, and the general transcriptional machinery. The mechanisms driving the formation of TF clusters, and their impact on transcription, however, remain largely unclear. This is mostly due to the lack of a tractable system. Here, we exploit the transcriptional activation of the mir430 locus in zebrafish embryos to simultaneously follow the clustering of Nanog, the underlying DNA, and transcription output. We show that Nanog binds to the mir430 locus to form clusters and that local DNA compaction result in the formation of a large dynamic TF-DNA cluster. Transcription begins only once this cluster reaches a critical amount of Nanog. While Nanog is not essential for initial DNA compaction, it stabilizes the cluster through its DNA-binding domain and intrinsically disordered regions. Our findings support a model in which Nanog clusters enhance gene activation by locally binding on DNA and stabilizing the TF/DNA cluster that forms by DNA compaction. This emphasizes the interdependent relationship between TFs and DNA dynamics in cluster formation. Together, these results suggest a mechanism in which transcription factor clustering, driven by local DNA interactions and compaction, acts as a regulatory hub to control the timing and robustness of gene activation during development.

Translation dynamics during zygotic genome activation

Mounia Lagha

Institut de Génétique Moléculaire

Visualisation of nuclear ncRNAs in living cells using Nuclear Background-reduced RNA-Imaging with Tamoxifen and ERT2 (Nuclear-BRITE)

Adam Cawte, Guifeng Wei, Tatyana Nesterova, Flavia Constantinescu, Mafalda Almeida, Neil Brockdorff

University of Oxford, Department of Biochemistry, South Parks Road, Oxford, OX1 3QU

The gold standard for imaging single molecules of RNA are bacteriophage derived labelling strategies such as, the MS2 system. However, when using these systems, RNA imaging within the nucleus is still challenging due to excess background fluorescence of nuclear localised MS2 capsid proteins. To address this, we developed a method to significantly reduce this excess by retaining the capsid proteins in the cytoplasm via fusing them with an ERT2 tag. This improves signal to noise ratios in the nucleus and allows for the accurate detection and tracking of single molecules at fast frame rates with super-resolved instant Structured Illumination Microscopy (iSIM).

With this technique, we can dissect the different diffusive behaviours of lncRNAs Xist and Malat-1, as well as short ncRNAs, U92 scaRNA, 5S rRNA and 7SK. As a proof-of-principle, we can image the lncRNA Xist at single-molecule resolution and visualise its spreading across the inactive X-chromosome in female mESCs. We observe that the majority of Xist particles are chromatin-associated, and their dynamics correlate with the underlying movements of chromatin and DNA-DNA contacts. The same is not observed for ncRNAs such as Malat-1 and U92 which show more diffusive behaviours with specific interactions with nuclear compartments that increase their residency times. Furthermore, 5S rRNA shows a strong nucleolar association consistent with its transcription and involvement in ribosomal biogenesis.

This methodology is fully compatible with endogenous tagging of RNA targets with no observed effect on function or localisation. The hope is that this methodology will enable direct visualisation of a wide range of nuclear ncRNAs and how they interact with nuclear compartments and chromatin to better understand their functions in the nucleus.

Dynamics of Gene Regulation, from the Nucleus to Mitochondria

Stirling Churchman

Harvard Medical School

The life cycle of RNAs are varied and diverse. To map the fate of RNA transcripts across the cell, we developed subcellular TimeLapse-seq, a technique that combines metabolic RNA labeling (4sU), subcellular fractionation, and nucleotide conversion chemistry to measure RNA flow rates across different cellular compartments. We measured how nuclear-encoded transcripts move through the cell by quantifying and modeling their half-lives on chromatin, in the nucleus, in the cytoplasm, and on polysomes. This revealed remarkable variations in RNA flow rates between genes and compartments. We identified genes that undergo substantial nuclear degradation by the exosome, and discovered associations between RNA-binding proteins and nuclear export rates. We also applied this approach to study mitochondrial gene expression, revealing striking differences between nuclear and mitochondrial transcripts. These studies provide comprehensive characterization of RNA life cycles throughout the cell and reveal previously unknown regulation mechanisms in both nuclear and mitochondrial gene expression.

PhD workshop pt.2

Stabilization of excised introns in yeast

Glenn Li (MIT/Whitehead Institute/HHMI),

Max Wilkinson (MIT/Broad Institute/HHMI), Alex Chan (MIT/Whitehead Institute), Ryan Muller (MIT/Whitehead Institute/HHMI), Nicholas Ingolia (University of California, Berkeley), Sebastian Lourido (MIT/Whitehead Institute), Gerald Fink (MIT/Whitehead Institute), David Bartel (MIT/Whitehead Institute/HHMI)

In *Saccharomyces cerevisiae*, prolonged cellular stress induces a subset of introns to accumulate post-splicing as stable, linear, spliceosome-protected RNAs (stable introns). These introns sequester splicing components, causing less efficient splicing in these stressed conditions. We determined a 2.9-Å-resolution cryo-EM structure of a stable intron purified from saturated culture conditions. This structure shows that stable introns are protected by a catalytically inactive Bact-like spliceosome. Using a genome-wide CRISPRi screen, we also identified genes required for stable-intron accumulation. For example, knockdown of components of the U1 snRNP or the Retention and Splicing complex affects the accumulation of excised stable introns without dramatically affecting pre-mRNA levels. The screen also identified Iks1 as a key regulator of stable-intron accumulation. Loss of Iks1 kinase activity causes depletion of stable introns, whereas perturbation of a putative autoinhibitory helix or overexpression of Iks1 causes stable introns to accumulate in log-phase growth, where they normally would not accumulate. Iks1 phosphorylates proteins involved in proteostasis, including the Hsp70 family members Ssa1–4. Hsp70 inhibition is sufficient to induce stable-intron expression, whereas disruption of a predicted physical interaction between Iks1 and Hsp70 reduces stable-intron expression. We propose that Iks1- and Hsp70-dependent regulation of spliceosomal components enables the spliceosome to assemble on linear excised introns and protect them from degradation during prolonged stress.

Large-scale organization of RNA-protein interaction networks reflects structure of the genetic code

Thomas H. Kapral & Bojan Žagrović

Max Perutz Labs & University of Vienna

Autogenous interactions between mRNAs and the proteins they encode are implicated in cellular feedback-loop regulation, but their extent and mechanistic foundation are unclear. It was recently hypothesized that intrinsic nucleobase–amino acid affinities may be related to the genetic code's structure and thus promote autogenous mRNA/protein interactions. Importantly, this premise could also contribute to rationalizing the large-scale structure of RNA-protein interaction networks, going beyond autogenous interactions. Leveraging our POSTAR3 CLIP-seq database, we report on widespread autogenous interactions across different organisms. Specifically, 230 of 341 (67%) studied RNA-binding proteins (RBPs) interact with their own mRNAs, with a heavy enrichment among high-confidence hits and a preference for coding sequence binding. We account for different confounding variables, including physical (overexpression and proximity during translation), methodological (difference in CLIP protocols, peak callers and cell types) and statistical (treatment of null backgrounds). Furthermore, by viewing RBPs through the lens of their own encoding transcripts, an unexpected simplicity emerges concerning the structure of the complete cellular RNA-protein interaction network. Specifically, there is a parallel between the composition of target mRNAs and the transcripts of the RBPs they bind: mRNAs rich in a given nucleotide are targeted by RBPs whose own transcripts are on average enriched in that nucleotide, and vice versa. This global pattern holds for

each of the four standard RNA nucleotides, is robust against outliers on both mRNA and protein side, and yields covariation measures of high statistical significance. Overall, our results reflect the close connection between coding and binding in biology and establish a general principle behind the large-scale structure of RNA-protein interaction networks: compositionally, an individual mRNA is an archetype of how its bound proteome is encoded.

TH Kapral, F Farnhammer, W Zhao, ZJ Lu & B Žagrović, "Widespread autogenous mRNA-protein interactions detected by CLIP-seq", *Nucleic Acids Research*, 50, 2022, <https://doi.org/10.1093/nar/gkac756>

W Zhao, S Zhang, Y Zhu, X Xi, P Bao, Z Ma, TH Kapral, S Chen, B Žagrović, YT Yang &

ZJ Lu, "POSTAR3: an updated platform for exploring post-transcriptional regulation coordinated by RNA-binding proteins", *Nucleic Acids Research*, 50, 2022, <https://doi.org/10.1093/nar/gkab702>

Deciphering the code: codons, codon-codon interactions, amino acids, and peptide bonds regulate mRNA stability

Haejeong Lee, Damir Musaev, Charles E. Vejnar, Srikar Krishna Gopinath, Ethan C. Strayer, Jean-Denis Beaudoin, Mario Messih, Antonio Giraldez

Yale University

During the translation process by the ribosome, reading the coding sequences (CDS) not only produces a peptide but also influences mRNA stability through codon optimality. However, codon optimality has been mainly studied at the level of individual codons. Furthermore, the sequence complexity of endogenous transcript has prevented us from disentangling codon specific effects from other features determining mRNA stability. To address this challenge and elucidate how codons, amino acids, codon pairs, and amino acid pairs collectively regulate mRNA stability, we designed a massively parallel reporter assay (MPRA) and measured reporter half-lives in both zebrafish embryos and human cells. To specifically deal with the translation-dependent RNA stability, we used reporter half-lives that are specific to the reading frame. Our results showed that the effect of individual codons cannot fully explain how CDS regulates mRNA stability. Instead, we found the effect of codons depends on the identity of the neighboring codons, and the order of these codons influences mRNA stability. Based on these results, we developed a pair-wise codon interaction matrix and a computational method to predict the effects of codon pairs on mRNA stability. Using it, we developed a computational method to design coding sequences optimized for stability and generated a luciferase mRNA that is more stable than the ones designed by other methods that only consider codon identity. We applied the algorithm to design CDS of clinically relevant RNA therapies to optimize their stability. Additionally, we observed amino acids and the order of the two adjoining amino acids influence mRNA stability, suggesting that peptide bond between specific amino acids regulates mRNA stability. Currently, we are investigating how evolutionary conservation relates to codon pair effects and how codon pair sequence influences mRNA translation using Nascent Peptide Translating Ribosome Affinity Purification (NaP-TRAP). These results will be presented at the meeting. Together, our comprehensive study on the regulation of mRNA stability by the CDS provides key insights into how codon pair interaction and peptide bond formation influence mRNA stability, which has important implications in RNA biology and therapeutics development.

PhD workshop pt.3

Live single-transcript imaging reveals global shift in particle dynamics during neuronal development

Aileen-Diane Bamford^{1,2,3}, Gilles Gut², Tim-Oliver Buchholz³, Ryoko Okamoto², Barbara Treutlein² and Franka Voigt¹

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³Friedrich Miescher Institute for Biomedical Research

The human brain is an extraordinarily complex structure, and its development relies on a carefully orchestrated series of events. While the robust spatiotemporal expression of core transcription factors has been established as a critical mechanism to ensure proper brain development, post-transcriptional mechanisms involved in gene regulation and cell type specification are less well understood. The complex shape of neurons allows them to form intricate networks and perform highly specialized and locally restricted functions, in some species on the scale of meters away from the cell's soma. This requires neuronal subcellular compartments, specifically neurites and spines, to act largely autonomously from the nucleus, responding rapidly to cues via redistribution and translation of a specific set of locally enriched mRNAs. To elucidate the role of global mRNA targeting on the maturation and proper function of neurons, we devised a live single molecule imaging approach that we apply in induced pluripotent stem cell (iPSC) – derived neuronal models. This allows us to record the spatiotemporal dynamics of mRNA localization at different stages of neurodevelopment and thus unveil the role of differential transcript distribution as a fundamental mechanism for gene expression regulation.

Specifically, we have developed a robust CRISPR/Cas9 gene editing workflow to integrate MS2 stem loop arrays into endogenous loci within human iPSCs and have generated a cell line that expresses fluorescently labeled MS2 coat proteins for mRNA visualization from one allele of the safe harbor locus AAVS1. We differentiate these genetically engineered human iPSCs into 3D neural organoids or inducible neurons in 2D culture and record mRNA mobility at different time points of development using a long working distance optical set-up that achieves single-transcript resolution throughout multiple layers of cells in opaque tissue.

We track and quantify localization and diffusion dynamics of transcripts encoding core cytoskeletal components, which contain known cis-regulatory elements in their 3' UTRs and are targeted for active transport in polarized cells, such as neurons. We chart mRNA behavior at different developmental stages, resolving dynamics in polarized neural progenitors, intermediates, and differentiated neurons. Strikingly, we find that transcript mobility patterns diversify as different cell types emerge, and tissue regions are stably established. We use Hidden Markov Modelling to infer underlying states and state switches within tracks and observe a substantial number of particles that are characterized by more than one type of motion, indicating a rapid switch between different diffusive phases or modes of transport. Based on the identified state switches, we cluster sub-tracks and correlate their hidden state with cytoskeletal association and sub-cellular compartments. The resulting map of sub-tracks serves as baseline for cluster enrichment/depletion analyses upon perturbations such as translation inhibition or disruption of the cytoskeleton. This enables us to quantify the contribution of different RNA targeting mechanisms to the global distribution of transcripts as a key regulatory step of post-transcriptional gene regulation during neuronal development.

Altogether, our approach allows for the tracking of thousands of mRNA particles, quantifying the dynamically changing motion of endogenous transcripts on the subcellular level and within tissues and thus provides new insights into the mechanisms and relevance of RNA localization during human brain development. Our versatile tool can easily be adapted to tag any endogenous transcript of interest in the large variety of cell types and tissues that can be derived from pluripotent stem cells.

Ashwin localizes the tRNA ligase complex to the nucleus for pre-tRNA splicing

Moritz Leitner

Max Perutz Labs & University of Vienna

The tRNA ligase complex (tRNA-LC) is essential in metazoa for the non-canonical splicing of intron-containing pre-tRNAs and the XBP1 mRNA during the unfolded protein response. The tRNA-LC was thought to be a pentameric complex, composed of RTCB – its catalytic subunit – and four additional subunits: the RNA helicase DDX1, FAM98B, CGI-99 and Ashwin (ASW). Here we unveil that additional forms of the tRNA-LC exist in human cells, where FAM98B is replaced by one of its paralogs, FAM98A or FAM98C. While all these tRNA-LC subpopulations show RNA ligase activity in vitro, only the FAM98B-containing tRNA-LC seems to be important for pre-tRNA splicing in cells. This essentiality is indirectly facilitated by the subunit ASW, which we found to assemble exclusively into FAM98B-containing tRNA-LC and to act as a transport factor, localizing the tRNA-LC to the nucleus, where pre-tRNA splicing occurs. This function is driven by two nuclear localization signals that are conserved within vertebrate ASW homologues. The specialisation of the FAM98 proteins and the binding of FAM98B to ASW likely evolved to provide the tRNA-LC with a simultaneous dual localization to catalyze the splicing of pre-tRNAs in the nucleus and the splicing of XBP1 mRNA in the cytoplasm.

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Session speakers day 3

Functional assembly and regulation of the RNA polymerase IV machinery in plants

Todd Blevins

Centre national de la recherche scientifique

Eukaryotes use RNA polymerase II (Pol II) to synthesize mRNAs from genes, allowing their translation into proteins for cellular functions. However, genomes also contain transposable elements (TEs) that can cause mutations when activated. Animals balance the need for gene transcription by Pol II against the risk of deleterious TE mobility by synthesizing small RNAs (piRNAs) that target and silence TEs. In plants, a specialized enzyme, RNA polymerase IV (Pol IV), transcribes TEs to produce small interfering RNAs (siRNAs) that guide DNA methylation to repress TEs. While the Pol IV complex is recruited to TEs via SNF2-like CLASSY (CLSY) proteins, how Pol IV partners with CLSYs remains mysterious. Here, we identified a conserved CYC-YPMF motif that is specific to Pol IV and positioned on the complex exterior. Furthermore, we found that this motif is essential for the co-purification of all four CLSYs with Pol IV, but that only one CLSY is present in any given Pol IV complex. These findings support a “one CLSY per Pol IV” model where the motif acts as a CLSY-docking site. Mutations in this site phenocopy pol iv null and clsy quadruple mutants. Together, these findings provide structural and functional insights into features that distinguish Pol IV from other RNA polymerases, allowing it to promote genome stability by targeting TEs for silencing.

Two phases of transposon regulation in *Drosophila* germline development

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Transposable elements (TEs) are major components of eukaryotic genomes and pose a significant threat to genome integrity. To counteract the detrimental effects of TE proliferation, host genomes have evolved small RNA-based transposon-silencing mechanisms, such as the Piwi-interacting RNA (piRNA) pathway in animals. While the genetic and molecular mechanisms of the piRNA pathway are well described, much of this knowledge is derived from experimentally tractable stages of germline development, such as late-stage oogenesis in *Drosophila*. However, the germline undergoes substantial chromatin remodeling and transcriptional regulation shifts throughout development, highlighting the need to explore how TEs and their repression systems adapt to these molecular changes.

In this study, we utilized the sophisticated genetic toolkit of *Drosophila melanogaster* to investigate the dynamic expression and regulation of TEs throughout germline development.

Our results reveal that upon disruption of the piRNA pathway, the expression of most TEs is specific to only the latest stages of germline development. Just a few TE families are expressed during earlier stages of germline development, including in primordial germ cells during larval stages. We also present evidence for a developmental switch in the expression of the piRNA defense genes and piRNA source loci, indicating that the piRNA pathway also adapts throughout germline development. Finally, we demonstrate that TEs can be transcriptionally silenced during the early stages of germline development, and this silencing is then maintained throughout development, even in the absence of functional piRNA defense. Building on these

findings, we propose that the timing of TE expression influences their regulation, with early TE expression contributing to more robust silencing throughout germline development. This could be one factor explaining why many TEs have evolved to be expressed only in the later stages of germline development, as this timing likely increase their chances of successful propagation.

Splicing regulation at the U2 snRNP-branchpoint complex

Douglas L. Black

*Department of Microbiology, Immunology, and Molecular Genetics
University of California, Los Angeles*

Understanding the mechanisms of pre-mRNA splicing and its regulation is limited by technical challenges to examining spliceosomes in vivo. I will present analyses of RNP complexes derived from precatalytic A or B-like spliceosomes isolated from the chromatin pellet of mammalian cell nuclei. We found that these complexes contain U2 snRNP proteins and a portion of the U2 snRNA bound with protected fragments of nascent RNA that precisely map to intronic branch sites across the transcriptome. These U2 complexes also contain the splicing regulators RBM5 and RBM10. We found RBM5 and RBM10 bind at nearly all branch site complexes and not simply those at regulated exons. Deletion of a conserved RBM5/RBM10 peptide sequence, including a zinc finger motif, disrupts U2 interaction and renders the proteins inactive for repression of many alternative exons. From these and additional structural studies, we propose a model where RBM5 and RBM10 regulate splicing as components of the U2 snRNP complex following branch site base-pairing.

tRNAslational control of eukaryotic gene expression

Sebastian Glatt

University of Veterinary Medicine Vienna & Jagiellonian University Krakow

My Research Group studies different translation control mechanisms, which regulate the production of specific sets of proteins by chemical modifications of tRNA molecules. Every protein in the cell is produced by the ribosome, which uses transfer RNA (tRNA) molecules to translate the sequence information coded in mRNAs into correctly assembled poly-peptide chains. The lab is focusing on understanding the molecular mechanisms that lead to the specific base modifications in anticodons of tRNAs. These modifications have a strong influence on the efficiency and accuracy of the codon-anticodon pairing and therefore regulate the translational rates and folding dynamics of protein synthesis. Recent findings have shown that alterations of these modification pathways play important roles in the onset of certain neurodegenerative diseases and cancer. We mainly use X-ray crystallography (MX) and cryogenic electron microscopy (cryo-EM) to obtain snapshots of the involved macromolecular machines and analyse their reaction intermediates at atomic resolution. Subsequently, we employ different complementary in vitro and in vivo approaches to validate and challenge our structural observations. We also have started working on other (t)RNA modification pathways and elucidate the structure of folded RNA molecules directly by cryo-EM. Furthermore, we aim to understand how these post-transcriptional modifications affect ribosomal decoding and translation elongation by directly imaging translating ribosomes at atomic resolution. Last but not least, we develop novel structural, biochemical and biophysical approaches to study structured RNA domains. In summary, our work contributes to the fundamental understanding of eukaryotic gene expression and its complex regulatory mechanisms.

High-Resolution Translation Landscapes in Human Cells

Wei Zheng¹, Yuekang Zhang¹, Jimin Wang¹, Shuhui Wang¹, Pengxin Chai¹, Elizabeth J Bailey¹, Wangbiao Guo^{2,3}, Swapnil C Devarkar¹, Shenping Wu⁴, Jianfeng Lin¹, Kai Zhang¹, Jun Liu^{2,3}, Ivan B Lomakin⁵, **Yong Xiong**¹

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Obtaining comprehensive structural descriptions of macromolecules within their natural cellular context holds immense potential for understanding fundamental biology and improving health. Here, we present the landscape of protein synthesis inside human cells in unprecedented detail obtained using an approach which combines automated cryo-focused ion beam (FIB) milling and in situ single-particle cryo-electron microscopy (cryo-EM). With this in situ cryo-EM approach we resolved high-resolution structures of the human 80S ribosome and unveiled over twenty distinct functional states. In contrast to in vitro studies, we identified protein factors, including SERBP1, EDF1 and NAC β , not enriched on purified ribosomes. Most strikingly, we observed that SERBP1 binds to the ribosome in almost all translating and non-translating states to bridge the 60S and 40S ribosomal subunits, suggesting that SERBP1 may serve an important regulatory role in translation. We also resolved high-resolution structures from cells treated with homoharringtonine and cycloheximide, and identified numerous polyamines bound to the ribosome, including a spermidine that interacts with cycloheximide bound at the E site of the ribosome. Collectively, our work underscores the importance of high-resolution in situ studies in the complex native environment.

A genome-wide screen reveals that oxidative stress regulates translation elongation to promote production of detoxifying selenoproteins

Frederick Rehfeld¹, He Zhang¹, Joshua Mendell²

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² Howard Hughes Medical Institute / University of Texas Southwestern Medical Center Dallas, United States of America

Selenocysteine (Sec), considered the 21st proteinogenic amino acid, is critical for catalysis by several enzymes that clear reactive oxygen species (ROS) from cells. As opposed to canonical amino acids, Sec is incorporated into the nascent peptide chain through recoding of a UGA termination codon. In eukaryotes, this event is specified by a cis-element in the 3' UTR of transcripts, termed SECIS, that is bound by RNA-binding protein SBP2, which in turn recruits the elongation factor EEFSEC and tRNA^{SEC} to the ribosome. Using GPX4, an essential glutathione-peroxidase and selenoenzyme as a Sec-reporter, we carried out the first genome-wide CRISPR-Cas9 knockout screen to comprehensively identify regulators of Sec decoding.

This screen successfully identified all known factors involved in the biosynthesis and incorporation of Sec. Intriguingly, among the most significant hits that increased Sec incorporation were canonical translation elongation factors. We could show that due to the naturally inefficient decoding of the Sec codon, GPX4 is subject to clearance by ribosome rescue pathways, activated by ribosome collisions. Reduction of canonical elongation factors and the concomitant global slowing of elongation, however, provided a window for enhanced Sec decoding. In addition, loss of thioredoxin reductase (TXNRD1) and of oxidative phosphorylation genes also increased Sec incorporation. Inactivation of these genes increased reactive oxygen species and perturbed the cellular redox balance. Interestingly, this also resulted in a slowdown of translation elonga-

tion. We identified the translation elongation factor EEF1 subunit gamma (EEF1G) as a cellular redox sensor that directly couples translation elongation rate to the redox state of the cell through a conserved glutathione-S-transferase (GST) domain. Thus, our data revealed a novel regulatory circuit that enables a rapid increase in the production of detoxifying Sec-containing proteins by globally slowing translation elongation upon exposure to oxidative stress.

Concentration matters: Regulation of ribosome homeostasis during cellular stress

Rachel Green, James A. Saba, Kate L. Schole, Frances Diehl

Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Ribosome concentration is carefully controlled to implement varied cellular programs. For example, neuronal cells and pancreatic cells have grossly different needs for protein synthesis as readily visualized through analysis of their polysome profiles. As such, signaling pathways mediated by ribosome collisions and kinases GCN2 and ZAK are expected to play different roles and have different thresholds for activation in these regimes. Driven by an interest in understanding the connections between ribosome homeostasis and ribosome-mediated signaling pathways, we are exploring the molecular determinants of ribosome homeostasis. We are developing methodology to carefully measure ribosome concentration, mRNA concentration, and the load of ribosomes on mRNAs in different cellular regimes. Our work thus far has converged upon two molecular controls of ribosome levels within cells. First, we have identified a role for LARP1 in directly sensing free 40S subunits to auto-regulate its own synthesis, and in turn, the production of r-proteins from TOP mRNAs. Second, we have defined mechanisms of ribosome degradation in cells responding to amino acid starvation and other stresses, mediated by the kinase R1OK3. These projects will be discussed in the context of the broader question of ribosome homeostasis.



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POSTER - SESSION 1 -

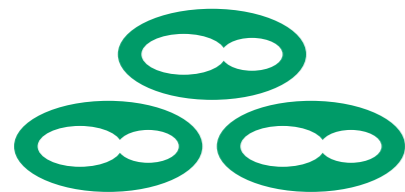
Wednesday, May 07

No	Name	Title
1	Maximilian Mager	The MTR4-NRDE2 Complex: Structural and Functional Implications in RNA Degradation
3	Katarina Gresova	miRBench: novel benchmark datasets for microRNA binding site prediction that mitigate against prevalent microRNA Frequency Class Bias
5	Selay Kaya	Consequences of targeted tRNA pool alterations for decoding speed and protein homeostasis in yeast
7	Max Graf	Two homologous complexes direct nuclear mRNPs towards export or degradation
9	Tajda Klobučar	Integrative profiling of condensation-prone RNAs during early development
11	Arantxa Rojas	HRDE-1 Mediates Germline Transcriptional Silencing Independently of H3K9me3 and Maintains Heritable Small RNA Pools
13	Andrea Gabriela Hernández Azurdia	Long-Term miRNA Silencing in Eggplant: ELVd-Based VIdGS Outperforms Traditional VIGS
15	Irene Stevens	THE EARLY TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL RESPONSES TO FLUCONAZOLE IN SENSITIVE AND RESISTANT CANDIDA ALBICANS
17	Kai Katsuya Gaviria	A Conserved Ribonuclease Cleaves an sRNA via a Conformational Switch: CryoEM Insights
19	Josie Maus-Conn	Investigating the functional divergence of Dicer's helicase domain
21	Göksu Bayrak	Using single-molecule imaging to investigate IRE1 oligomerization during XBP1 splicing and mRNA decay
23	Felix Fischer	From genetic code to phase separation: How composition shapes RNA-protein interactions
25	Xavier Rambout	Competition between pre-mRNA stabilization and decay pathways regulates mitochondrial function in development and disease
27	Simon Aschenwald	Deciphering the Molecular Mechanism underlying Cluster Assistance in miRNA Biogenesis
29	Fatima Berro	Unveiling the silence: how translation quiescence shapes mRNA stability in oocytes
31	Suria Itzel Morales Guzman	Investigating mRNA targeting mechanisms and functional relevance of Dync1h1 granules in mouse intestinal organoids using single-molecule imaging.
33	Arjun Udupa	A comprehensive interrogation of transcription factors in mouse embryonic stem cells
35	Malvin Leonardo Pardi	SARS-CoV-2 Nsp1 interacts with stalled ribosomes and promotes stall read-through
37	Khadija Hajji	The edited isoform of the Drosophila Adar RNA editing enzyme regulates ecdysone signalling and innate immunity during development
39	Sakshi Gorey	Regulation of protein abundance in neurons by selective translation of 3'UTR isoforms
41	Ming Yang	Investigating the molecular mechanisms underlying IRE1-dependent mRNA turnover on the ER
43	Magda Kopczyńska	Histone methyltransferase SETD2 regulates transcription termination by suppressing cryptic initiation and enhancing 3' pre-mRNA cleavage
45	Julieta Mateos	COLD-INDUCED PHASE SHIFT OF MIR157 EXPRESSION MODULATES FLOWERING AND ROOT ARCHITECTURE IN ARABIDOPSIS
47	Julian Schoth	Investigating conserved RNA elements as novel drug targets for antiviral therapy
49	Ewa Stein	Sf insect cell lines culturing for purification of mammalian factors required for the pre-mRNA 3'-end maturation
51	Klara Kuret Hodnik	More than a splicing factor: exploring the cellular functions of ZNF830
53	Katarzyna Speichert	Centriolar satellites are sites of translation of centrosomal and ciliary proteins
55	Josef Roehsner	Deciphering regulation and functional conservation of dormant ribosomes
57	Iori Morita	Computational design of a programmable sequence-specific ssRNA binding protein
59	Jure Rebselj	Role of multivalent nucleotide sequences in mRNA coregulation
61	Luca Gál	Exosomal small RNA profiling in first-trimester maternal blood explores early molecular pathways of preterm preeclampsia
63	Bernadett Torner	Functional role and diagnostic application of differentially expressed miRNAs in Lung Adenocarcinoma Brain Metastasis and Glioblastoma.
65	Zsuzsanna Birkó	Identification of deregulated miRNAs and mRNAs involved in glioblastoma development using next-generation RNA sequencing
67	Jakub Nowak	DyRNA Thermometry allows to monitor position specific structural changes in folded RNAs
69	Simon Bressendorff	Importance of an N-terminal structural switch in the distinction between small RNA-bound and free ARGONAUTE
71	Praveenkumar Rengaraj	Tackling the molecular function of human RNA demethylase ALKBH5
73	Aidan Tollervey	Investigating RNA-RNA interactions in rotavirus genomic packaging
75	Neža Vadnjal	The role of interstasis in regulation of multivalent mRNAs and low-complexity domain containing proteins
77	Alexandra Wagner	Spike-in normalization enhances reliability of identifying CSF-enriched miRNAs by small RNA-sequencing
79	Wiktoria Płonka	Differential impact of DGCR8 silencing on microRNA biogenesis in lymphoma cells
81	Malgorzata Rak	miR-652-3p target genes are involved in controlling of pro-inflammatory cytokines and autophagy in ischemic stroke
83	Gabriela Chodorowicz	Distinguishing diabetic ischemic stroke: The role of serum exosomal miR-652-3p

POSTER - SESSION 2 -

Thursday, May 08

No	Name	Title
2	Markus Landthaler	Investigating the functional roles of eukaryotic translation initiation factors through rapid protein degradation and ribosome profiling
4	Alok Tiwary	Gametocyte Specific Factor 1 (GTSF1) in PIWI Protein Functions
6	Juan Camillo Diez Marluanda	Interplay between salicylic acid and small RNAs in plant stem cell antiviral defense
8	Jonas Schrade	The role of the endonuclease Zucchini in piRNA biogenesis in pluripotent stem cells of planarians
10	Emilia Arguello	Probing RNA Structure With X-Rays Via XFP-Seq
12	Marine Navarro	Fungal microRNAs: key players in interaction
14	GEETIKA BAJAJ	MicroRNAs Dependent G-ELNs Based Intervention Improves Glucose and Fatty Acid Metabolism while Protecting Pancreatic β -Cells in Type 2 Diabetic Mice
16	Baptiste Rafanel	Taming an endogenous retrovirus
18	Daphne Jurriens	Optical tweezers and single molecules: control, manipulate and visualize protein-RNA interactions and conformations in real-time
20	Laura Hertz	High-throughput evolution evidence for the mechanism of the fluoride ribo-switch
22	Marlene Adlhart	Coding relationship links RNA G-quadruplexes and protein RGG motifs
24	Shamitha Govind	From PIWI to RdRP: Functional Divergence of GTSF-1 in Nematodes
26	Denisa Jansová	Uncovering the Secrets of RNA Localization in Female Infertility
28	Weronika Sokolowska	Modulation of miRNA Biogenesis by Ionizing Radiation in Burkitt Lymphoma Cells
30	Changwei YU	Nascent RNA Decay via the Nuclear Exosome is Essential for Piwi-Mediated Transposon Silencing
32	Hania Paola Leon Contreras	sRNA biogenesis in duckweed: the transitivity dilemma
34	Marieke Trasser	Germline small RNA modification in epigenetic inheritance
36	Viveka Viveka	Upstream cues regulating Alternative splicing (AS) of auxin-dependent processes
38	Andrea Rodi	Elucidating the role of nuclear compartmentalization in testis-specific gene regulation
40	Nikolaus Beer	Developing models to visualize translational heterogeneity and RNA localization biases in live intestinal epithelium
42	Marlene Reichel	ALBA proteins facilitate cytoplasmic YTHDF-mediated reading of m6A in Arabidopsis
44	Iskra Sainova	Investigation the role of mitochondria in the processes of differentiation of human mesenchymal stem/progenitor cells, mouse embryonic 3T3 fibroblasts and mouse embryonic stem cells
46	Reejuana Parveen	Potential role of AAA+ proteases in ribosome turnover
48	Joanna Kwiatkowska	In vitro reconstitution of active pre-mRNA 3'-end cleavage and polyadenylation machinery
50	María García Gallardo	Activation kinetics of promoters in their interaction with enhancer elements.
52	Ewa Gutmajster	Studying possible differences in functions of LINC00116 or LINC00116-encoded Mitoregulin peptide in B-cell lymphoma cells
54	Julia Corell Sierra	Uncovering tsRNA Roles in Plant Stress: A Bioinformatic Exploration Across Species
56	Antonio González Sánchez	Global analysis of stress response mediated by miRNAs in plants
58	Radvilas Bendorys	Sequence-Specific DNA Hydrolysis and Partial Reverse Splicing by an ORF-Less Bacterial Group IIC Ribozyme In Vitro
60	Savani Anbalagan	Gas-sensing riboceptors
62	Urska Janjos	Assessing the function of conserved cryptic splice sites in Hnrnp1 introns
64	Alexander Hammermeister	Structural characterization of tRNA binding by the human FTSJ1-THADA methyltransferase complex
66	Luka Gnidovec	Collective dosage regulation of condensation-prone proteins influences cellular physiology and proliferation
68	Shwetha Krishna	Adenosine (de)methylation on the crossroads of RNA and DNA synthesis
70	Veronika Rájecká	Global analysis by LC-MS/MS of N6-methyladenosine and inosine in mRNA reveal complex incidence
72	Veroni de Ree	RNAi induced gene silencing via poly(UG) tailed RNA in the satellite model organism <i>Pristionchus pacificus</i>
74	Lalitha Veleti	piRNA pathway beyond <i>Drosophila</i> : Argonaute expansion in <i>Bradysia</i>
76	Marianne Pultar	Preserving the Message: isomiR Landscapes in Cells, EVs, and Protein Complexes
78	Ádám Csók	Alterations of miRNA expression in metastatic Wilms' tumor
80	Tibor Csorba	Transcription fidelity is needed for development and heat stress survival in <i>Arabidopsis</i>
82	Nikolaos Balatsos	Circadian expression and time-of-day interacting partners of poly(A)-specific ribonuclease
84	Hana Marvanova	The door opens only for those who have already walked through it: mechanistic dissection of the parent-of-origin effect in the toxin-antidote element <i>slow-1/grow-1</i>



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Poster Abstracts

POSTER NUMBER: 1

The MTR4-NRDE2 Complex: Structural and Functional Implications in RNA Degradation

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RNA degradation plays a crucial role in maintaining RNA homeostasis and regulating gene expression, making it a key aspect of cellular RNA metabolism. In the nucleus, the exosome interacts with cofactors like MTR4, which contributes to the degradation of RNA. Proteins associated with MTR4 affect RNA transcript selectivity, and one such protein might be NRDE2. The aim of this study is to uncover the functional role of the interaction between NRDE2 and MTR4 in the nuclear RNA degradation pathway. Although MTR4 is a well-studied DExH-box RNA helicase that promotes RNA degradation as a crucial cofactor to the nuclear RNA exosome, the role of NRDE2 in this process is less clear. While NRDE2 has previously been described as a negative regulator of RNA degradation [1], our findings suggest that it may play a role in promoting RNA degradation in the nuclear exosome pathway. This discrepancy highlights the need for further investigation to clarify NRDE2's function in the nuclear RNA degradation pathway. Using cryo-electron microscopy, molecular dynamics simulation and biochemical assays, we examine the structural and functional roles of the MTR4-NRDE2 complex. Biochemical analysis revealed the stimulating function of NRDE2 on MTR4's ATPase activity, highlighting the complex's role as a novel RNA-targeting factor. Previous work has also highlighted NRDE2's involvement in pre-mRNA splicing [2], [3], suggesting a potential functional link between pre-mRNA splicing and degradation pathways that we aim to explore further. Additional research is needed to fully understand the complex regulation of RNA degradation at different stages of RNA processing and to clarify how interactions between MTR4 and NRDE2 influence these processes.

References:

- [1] J. Wang et al., "NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction," *Genes Dev.*, vol. 33, no. 9–10, pp. 536–549, May 2019, doi: 10.1101/gad.322602.118.
- [2] M. Flemr et al., "Mouse nuclear RNAi-defective 2 promotes splicing of weak 5' splice sites," *RNA*, vol. 29, no. 8, pp. 1140–1165, Aug. 2023, doi: 10.1261/rna.079465.122.
- [3] P. Richard et al., "NRDE-2, the human homolog of fission yeast Nrl1, prevents DNA damage accumulation in human cells," *RNA Biology*, vol. 15, no. 7, pp. 868–876, Jul. 2018, doi: 10.1080/15476286.2018.1467180.

POSTER NUMBER: 2

Investigating the functional roles of eukaryotic translation initiation factors through rapid protein degradation and ribosome profiling

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Protein synthesis is primarily regulated at the translation initiation step, which involves the dynamic assembly of the translation initiation machinery and is influenced by various features of the 5' leader. Although translation initiation has been studied extensively, the role of individual proteins in the process has not been fully elucidated. Most eukaryotic translation factors are essential and their removal through CRISPR or RNAi severely disrupts translation and homeostasis, making direct effects practically indistinguishable from indirect effects. Here, we use an auxin-inducible degron (AID) system to acutely and rapidly deplete the translation initiation factors EIF4E, EIF4G1, and EIF3D in human cells. We investigate the functions of these key translation factors by using ribosome profiling to monitor ribosome occupancy and translation efficiency across transcripts. Furthermore, we use a random forest model to identify transcript features that are important for predicting the effect of a factor's depletion on transcript translation efficiency.

POSTER NUMBER: 3

miRBench: novel benchmark datasets for microRNA binding site prediction that mitigate against prevalent microRNA Frequency Class Bias

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MicroRNAs (miRNAs) are crucial regulators of gene expression, but the precise mechanisms governing their binding to target sites remain unclear. A major contributing factor to this is the lack of unbiased experimental datasets for training accurate prediction models. While recent experimental advances have provided numerous miRNA-target interactions, these are solely positive interactions. Generating negative examples in silico is challenging and prone to introducing biases, such as the miRNA frequency class bias identified in this work. Biases within datasets can compromise model generalization, leading models to learn dataset-specific artifacts rather than true biological patterns. We introduce a novel methodology for negative sample generation that effectively mitigates the miRNA frequency class bias. Using this methodology, we curate several new, extensive datasets and benchmark several state-of-the-art methods on them. We find that a simple convolutional neural network model, retrained on some of these datasets, is able to outperform state-of-the-art methods. This highlights the potential for leveraging unbiased datasets to achieve improved performance in miRNA binding site prediction. To facilitate further research and lower the barrier

to entry for machine learning researchers, we provide an easily accessible Python package, miRBench, for dataset retrieval, sequence encoding, and the execution of state-of-the-art models.

POSTER NUMBER: 4

Gametocyte Specific Factor 1 (GTSF1) in PIWI Protein Functions

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PIWI proteins, a specialized class of Argonaute proteins, use 21–35 nucleotide PIWI-interacting RNAs (piRNAs) as guides to find and cleave RNA from transposons and other selfish genetic elements, thereby maintaining the integrity of the germline genome¹. Recent studies show that Gametocyte specific factor 1 (GTSF1) and its paralogs act as auxiliary factors to activate the RNA endonuclease activity of the cytoplasmic PIWI proteins MIWI and MILI2 in mice and Siwi and BmAgo3 in silk moth cells^{2,3}. GTSF1 is also required for the function of the nuclear PIWI proteins MIWI2 in mouse and Piwi in *Drosophila melanogaster*. Unlike cytoplasmic PIWI proteins, nuclear PIWIs are believed to use piRNAs to bind nascent transposon transcripts and then recruit DNA- or histone-modifying enzymes, converting the adjacent DNA to heterochromatin^{4,5}. How GTSF1 proteins accelerate target cleavage or enable DNA heterochromatinization remains poorly understood. Why do PIWI proteins need GTSF1 for their functions?

In mice, immobilized GTSF1 can capture all the three PIWI proteins from testis lysate, but in HEK293T cells, co-expressed GTSF1 and PIWI proteins do not co-immunoprecipitate, suggesting a role for other RNAs or proteins in GTSF1–PIWI interactions⁵. We have generated *D. melanogaster* lines in which each of the four GTSF1 paralogs is epitope tagged. Immunofluorescence studies show different spatial localization of the paralogues in *D. melanogaster* germline suggesting they have distinct functions. Using co-immunoprecipitation and mass spectrometric analysis of the tagged GTSF1–piRNA RISC complex, we seek to (1) determine if there is a one-to-one association between a PIWI protein and its auxiliary GTSF1 paralogue; and (2) identify and characterize the additional factors important for the formation and function of the GTSF1•piRNA•PIWI protein complex².

References

1. Ozata, D. M., Gainetdinov, I., Zoch, A., O'Carroll, D. & Zamore, P. D. PIWI-interacting RNAs: small RNAs with big functions. *Nature Reviews Genetics* vol. 20 89–108 (2019).
2. Arif, A. et al. GTSF1 accelerates target RNA cleavage by PIWI-clade Argonaute proteins. *Nature* 608, 618–625 (2022).
3. Izumi, N., Shoji, K., Kiuchi, T., Katsuma, S. & Tomari, Y. The two Gtsf paralogs in silkworms orthogonally activate their partner PIWI proteins for target cleavage. (2023) doi:10.1261/rna.
4. Dönertas, D., Sienski, G. & Brennecke, J. *Drosophila* Gtsf1 is an essential component of the Piwi-mediated transcriptional silencing complex. *Genes Dev* 27, 1693–1705 (2013).
5. Yoshimura, T. et al. Mouse GTSF 1 is an essential factor for secondary piRNA biogenesis. *EMBO Rep* 19, (2018).

POSTER NUMBER: 5

Consequences of targeted tRNA pool alterations for decoding speed and protein homeostasis in yeast

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The differential usage of synonymous codons plays a major role in setting the non-uniform pace of translating ribosomes along different mRNAs. This can impact the folding of nascent proteins, many of which begin to acquire their native structure in concert with their synthesis. How tRNA abundance modulates the ribosome's pace and protein homeostasis is poorly defined. To address these questions, we analyzed the relationship between tRNA availability and codon translation rates in budding yeast. Using high-resolution ribosome profiling, we found that ribosome occupancy at rare codons in endogenous mRNAs decreases in cells overexpressing matching low-abundance tRNAs, and increases in cells lacking non-essential rare tRNAs. Unexpectedly, some of these targeted tRNA pool manipulations altered ribosome dwell times also at near-cognate codons through tRNA sequestration. In all cases, tRNA abundance alterations had disproportionately large effects on ribosome occupancy at specific instances of the affected codons, which did not depend on flanking mRNA or protein sequences. Surprisingly, the overproduction of rare or non-native tRNAs had little discernible effects on cell fitness under normal conditions despite accelerated decoding, but it impaired the stress resistance of cells lacking specific translation quality control factors. Our findings demonstrate the challenges to predicting the functional consequences of tRNA abundance variations and emphasize the complexity of the rules governing the regulation of protein biogenesis in cells.

POSTER NUMBER: 6

Interplay between salicylic acid and small RNAs in plant stem cell antiviral defense

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RNA interference (RNAi) confers antiviral immunity to plants by generating virus-derived small interfering RNAs that are used to target viral RNA for degradation or translational repression, decreasing the accumulation of virus in the plant (Lopez-Gomollon & Baulcombe, 2022). Salicylic acid (SA), a defense-related phytohormone, is also involved in response against pathogens, mainly by inducing systemic acquired resistance (Peng et al., 2021), but also by activating RDR1 (Hunter et al., 2013), a key protein for antiviral immunity. Surprisingly, it is common for stem cells, located in the shoot apical meristem, to remain virus-free during viral infection (Bradamante et al., 2021). While this phenomenon has been known for a long time, the mechanism by which it happens is only partially understood. Incarbone et al. (2023) demonstrated that RDR1 mediates the exclusion of Turnip mosaic virus (TuMV) from stem cells in a SA-dependent manner. However, broadly speaking, RDR1 regulation and SA-mediated immunity activation upon viral infection are still poorly understood. Hence, this work aims to understand the interplay between RDR1 and SA during viral infection, and how this leads to virus exclusion from the meristem, along with the possible activation of other antiviral pathways. Here, we show that the expression of several genes of *Arabidopsis thaliana* during infection with

different viruses is affected in a SA-dependent manner, suggesting possible SA-dependent antiviral/proviral pathways that have not been extensively studied before. In addition, we find stem cell exclusion of TuMV to be NPR1- and NPR3/NPR4-independent. An important focus of our work is to identify possible protein and RNA interactors of RDR1. To this end, RDR1 was successfully immunoprecipitated for the first time. Yeast two hybrid (Y2H) screening, IP-MS, and proximity labelling are being used to assess RDR1 interactome. An Arabidopsis protein was determined as a possible interactor. In summary, our work on SA and RDR1 promises to uncover novel antiviral pathways that will hopefully provide new tools to combat disease in plants.

POSTER NUMBER: 7

Two homologous complexes direct nuclear mRNPs towards export or degradation

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During eukaryotic gene expression, nuclear mRNAs associate with proteins to form mRNPs, which are then exported to the cytoplasm for translation. However, gene expression often produces aberrant mRNPs, that must instead be targeted for degradation for faithful gene expression. While there is a better understanding how functional mRNPs are exported, it remains unclear how aberrant mRNPs are recognized and targeted for degradation.

The processing of mRNAs in the nucleus results in the deposition of distinct molecular marks, that are read out in the mRNA export pathway to ensure that only functional transcripts are transported to the cytoplasm for translation. A central player in this process is the DECD box ATPase UAP56, which facilitates mRNP licensing and docking at the nuclear pore complex (NPC) through its interaction with TREX-2 (1). The TREX-2 complex, comprising at its core GANP-PCID2-SEM1, triggers the release of RNA from UAP56 to facilitate export (1). We show that the LENG8-PCID2-SEM1 complex exhibits structural and biochemical similarity to TREX-2, binding to UAP56, stimulating the apparent ATPase rate of UAP56 and promoting UAP56 unclamping from RNA. Through knockout-rescue experiments using targeted LENG8 mutants, we demonstrate that these molecular functions are essential for in vivo activity. Strikingly, we find that LENG8-PCID2-SEM1 directly associates with ZFC3H1, a core component of the PAXT connection. PAXT links to the nuclear exosome and is essential for the degradation of faulty nuclear polyadenylated RNAs (2). Loss of the LENG8 – ZFC3H1 interaction leads

to the accumulation of various polyadenylated RNAs, suggesting that LENG8-PCID2-SEM1 functions as a key factor in nuclear RNA fate determination.

Taken together, our results reveal a conserved mechanism by which nuclear mRNPs are targeted for either export or decay. We propose that UAP56 serves as a molecular mark that is read out by two competing complexes - TREX-2 for nuclear export and LENG8-PCID2-SEM1 for degradation. The TREX-2 and LENG8-PCID2-SEM1 complexes therefore might play a role in maintaining nuclear mRNA homeostasis by targeting overlapping sets of transcripts representing a novel regulatory step in nuclear gene expression.

References

1. Hohmann, U. et al. A molecular switch orchestrates the nuclear export of human messenger RNA. bioRxiv 2024.03.24.586400 (2024) doi: 10.1101/2024.03.24.586400v2.
2. Meola, N. et al. Identification of a Nuclear Exosome Decay Pathway for Processed Transcripts. Mol Cell 64, 520–533 (2016).

POSTER NUMBER: 8

The role of the endonuclease Zucchini in piRNA biogenesis in pluripotent stem cells of planarians

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The planarian flatworm *S. mediterranea*, that consists of up to a quarter of pluripotent stem cells termed neoblasts, is an established model organism in the field of regenerative biology. Neoblasts contain large amounts of active transposable elements (TE) that, apart from being a result of stem cell plasticity, pose a great risk of disrupting essential genes. To counteract this potentially detrimental effect, a potent RNA based pathway based on small non-coding RNAs, piRNAs, bound to PIWI proteins, is indispensable for neoblast function. In general, there are two routes for the biogenesis of piRNAs, termed primary and the secondary piRNA biogenesis pathways. For primary biogenesis, piRNA precursors must be cleaved into smaller units (pre-piRNAs) before their further 3'-end resection results in mature piRNAs. The endonuclease, that carries out this cleavage step, is called Zucchini, an instrumental player in the primary piRNA pathway. Despite the importance of Zucchini for piRNA biogenesis in *Drosophila* and mouse, its exact role in *S. mediterranea* is unclear to date. This project therefore aims to overcome this knowledge gap.

To better understand the importance of Zucchini for planarian piRNA biogenesis, I first conducted Zucchini knockdown experiments by use of RNA interference. Very surprisingly, the efficient knockdown neither showed phenotypical changes nor lethality during regular homeostasis, as well as under conditions requiring regeneration. Moreover, I did not detect altered mature piRNA levels following UREA-PAGE analysis. These results argue that, in planarians, secondary piRNA biogenesis dominates overall mature piRNA levels. To investigate this hypothesis further, all piRNA biogenesis in planarians was attempted to be knocked down by co-depleting Armitage, an essential component of all piRNA biogenesis in *Drosophila* and mouse and by measuring its effect on viability, regeneration and piRNAs levels. Currently, I am investigating the consequences of Zucchini depletion on a transcriptomic level. For this purpose, neoblasts are sorted at two time points following Zucchini knockdown experiments using FACS (fluorescence activated cell sorting). The expected sequencing results from these neoblasts will reveal how a Zucchini knockdown perturbs the primary piRNA biogenesis pathway. In more detail, I will also learn how a Zucchini knockdown

influences the piRNA precursor cleavage patterns. In summary, I expect the result of this project to clarify the role of Zucchini in primary piRNA biogenesis in planarian stem cells, and to rationalize the surprising lack of lethality of a Zucchini knockdown for the animal.

POSTER NUMBER: 9

Integrative profiling of condensation-prone RNAs during early development

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Complex RNA–protein networks play a crucial role in the formation of many types of biomolecular condensates. How intrinsic RNA features contribute to condensate formation however remains unclear. Here, we integrate tailored transcriptomics assays to identify a distinct class of developmental condensation-prone RNAs termed ‘smOOPs’ (semi-extractable, orthogonal organic phase separation-enriched RNAs). These transcripts are localised to larger intracellular foci, form denser RNA-RNA interaction subnetworks than expected and are heavily bound by RNA binding proteins (RBPs). Using an explainable deep learning framework, we reveal that smOOPs harbor characteristic sequence composition, increased intramolecular folding and specific RBP binding patterns. Intriguingly, these RNAs also encode for proteins that contain intrinsically disordered regions and are predicted to be involved in biomolecular condensates, indicating an interplay between RNA- and protein-based features in phase separation. This work advances our understanding of condensation-prone RNAs and provides a versatile resource to further investigate RNA-driven condensation principles.

POSTER NUMBER: 10

Probing RNA Structure With X-Rays Via XFP-Seq

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Many RNA viruses rely on structured RNA elements to influence cellular machinery, evade immune detection, and replicate. Therefore, understanding the structure of viral RNA is essential for unraveling the mechanisms that govern viral infection. RNA structure probing has been crucial for understanding how viral RNA folds and interacts with self or host elements at various stages of the viral life cycle. Traditional chemical structure probing relies on mutational signatures induced by small molecules on “flexible” RNA regions but often operates on long timescales and can disrupt native RNA-biomolecule complexes, making in situ RNA structure probing difficult. Here, we introduce a novel method for determining RNA structure using X-ray footprinting combined with next-generation sequencing (XFP-Seq). This technique maps the solvent accessibility of the RNA backbone using X-ray-induced hydroxyl radicals on a millisecond timescale, introducing cleavage sites detectable at nucleotide-level resolution through sequencing. We also develop a new computational pipeline for processing and analyzing XFP-Seq data. We demonstrate the efficacy of XFP-Seq in fragmenting RNA and determining solvent accessibility by probing rotavirus RNA segments and mapping the global structural

changes occurring within viral condensates. Our approach addresses the temporal and chemical limitations of traditional RNA structure probing and will aid in identifying key RNA elements that play essential roles in rotavirus replication. Ultimately, we aim to tackle more complex challenges in structure probing, such as determining transient RNA motifs in condensate maturation, rationally designing next-generation RNA therapeutics, and 3D RNA modeling.

POSTER NUMBER: 11

HRDE-1 Mediates Germline Transcriptional Silencing Independently of H3K9me3 and Maintains Heritable Small RNA Pools

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Small RNAs and their associated Argonaute proteins were first identified in the phenomenon of RNA interference (RNAi), where they induce gene silencing through base complementarity, either post-transcriptionally in the cytoplasm or co-transcriptionally in the nucleus. RNAi is heritable in the nematode *Caenorhabditis elegans*, and many of the factors involved in its initiation and inheritance have been elucidated. Notably, the germline-specific nuclear Argonaute HRDE-1 (Heritable RNAi Deficient-1) was the first factor identified as essential for RNAi inheritance. However, recent studies have shown that RNAi inheritance can occur independently of HRDE-1 and even in a nuclear-independent manner (PMID: 35739318, PMID: 37878696). These findings challenge the prevailing model that HRDE-1 exclusively instructs heritable silencing. Furthermore, although RNAi-mediated transcriptional silencing correlates with the deposition of repressive histone modifications such as H3K9me3, the mechanism by which HRDE-1 represses endogenous targets remains unclear. To address this, I employed an Auxin-Inducible Degron (AID) system combined with germline nuclei sorting to transiently degrade HRDE-1 and track its regulatory dynamics during germline development and across generations. Using this approach, I identified a subset of HRDE-1 targets that are transcriptionally silenced in the germline. Surprisingly, their de-silencing occurs without loss of H3K9me3. In addition, some of the de-silenced genes acquire H3K4me3. Despite this de-silencing, small RNA levels initially remain unchanged, but gradually decline over several generations, suggesting a transgenerational role for HRDE-1 in maintaining heritable small RNA levels in the cytosol. Consistent with this, I observed that the re-establishment of silencing depends on the levels of heritable small RNAs.

Overall, our study demonstrates that HRDE-1 is essential for transcriptional repression of a specific subset of targets independently of H3K9me3, and also plays a critical role in transgenerational small RNA maintenance required for silencing re-establishment.

POSTER NUMBER: 12

Fungal microRNAs: key players in interaction

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INRAE

The *Fusarium* genus comprises over 80 phytopathogenic species responsible of Fusarium Head Blight, or FHB, a devastating cereal disease worldwide. FHB causes significant economic losses and causes health problems due to the production of mycotoxins by fungi, thermostable molecules dangerous to humans and animals, if ingested. FHB results from the interaction of several *Fusarium* species, including up to sixteen different species. Recent studies have shown that the infection stage and mycotoxin production are influenced by intra-microbial interactions mediated by molecular dialogue. We hypothesized that this molecular dialogue is mediated by microRNAs (miRNAs). MiRNAs are small non-coding RNAs, of 18–25 nucleotides, that induce post-transcriptional gene silencing. The abundance and nature of miRNAs are influenced by various factors such as stress, developmental stages, and microbial interactions. The aim of our research is to explore miRNAs of *Fusaria* during interactions. To achieve this, confrontation tests between *Fusarium graminearum* and other *Fusaria* were set up to investigate interaction zones. Morphological modifications were initially observed using confocal microscopy, which raises questions about the mechanisms behind these morphological changes. Multi-omics approach was realized, including comparative transcriptomic analyses to identify miRNAs and their potential targets during *Fusaria* interactions. In addition, metabolomic analyses using HPLC-MS complemented this approach to detect signature metabolites of the interaction. These results will give us keys to the diagnosis and prevention of FHB and mycotoxin accumulation in the plant system.

POSTER NUMBER: 13

Long-Term miRNA Silencing in Eggplant: ELVd-Based VldGS Outperforms Traditional VIGS

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MicroRNAs (miRNAs) play a crucial role in regulating plant responses to environmental stresses. Among them, miR398 and miR408 are key modulators of copper homeostasis and oxidative stress responses, targeting genes such as CSD1, CSD2, LAC, and PCY. Understanding the functional relevance of these miRNAs under stress conditions is essential for improving crop resilience. In this study, we employed the Viroid-Induced Gene Silencing (VldGS) approach using Eggplant Latent Viroid (ELVd) to silence miRNA precursors in eggplant (*Solanum melongena*). This method exploits the unique ability of ELVd-derived small interfering RNAs (siRNAs) to translocate into the nucleus, enabling efficient and long-lasting gene silencing.

Agroinfiltration of eggplant seedlings with constructs carrying ELVd-miR398 and ELVd-miR408 enabled spe-

cific silencing of these miRNAs. As an alternative silencing control, a modified Tobacco Rattle Virus (TRV) system (TRV-miR398 and TRV-miR408) was also employed. Phenotypic analysis under drought stress conditions revealed that eggplants infected with ELVd or TRV alone exhibited enhanced drought tolerance, maintaining better water status and reduced oxidative damage compared to ELVd-miR398- and ELVd-miR408-infected plants. Gene expression analysis confirmed that ELVd-miR398 plants showed reduced CSD1 and CSD2 transcript levels, while ELVd-miR408 plants exhibited lower LAC and PCY expression, correlating with impaired oxidative stress responses and copper metabolism.

Importantly, VldGS-mediated silencing persisted for at least 60 days post-infection, significantly outlasting conventional virus-induced gene silencing (VIGS), which typically lasts 15–20 days. Additionally, viral genomes encode multiple proteins that can disrupt plant homeostasis by interfering with endogenous cell mechanisms, representing a key disadvantage of VIGS compared to VldGS. This extended silencing period underscores the potential of VldGS as a stable and efficient tool for functional genomics in horticultural crops. Furthermore, our findings provide direct evidence of siRNA-mediated nuclear transport for miRNA silencing, expanding the utility of viroid-based tools in plant molecular research.

Overall, our study highlights the essential role of miR398 and miR408 in drought tolerance, likely through their regulation of reactive oxygen species (ROS) scavenging and electron transport. By demonstrating the effectiveness of ELVd-based VldGS, we propose this method as a valuable approach for dissecting gene function and enhancing stress resilience in crops. These findings pave the way for future applications of viroid-derived systems in functional genomics and plant biotechnology.

POSTER NUMBER: 14

MicroRNAs Dependent G-ELNs Based Intervention Improves Glucose and Fatty Acid Metabolism while Protecting Pancreatic β -Cells in Type 2 Diabetic Mice

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Metabolic disorders such as Type 2 diabetes mellitus (T2DM) pose a significant global health challenge. Exosomes are small extracellular vesicles secreted by all cell types and are known to mediate intercellular communication in both health and disease. Further, plant-derived exosome-like nanoparticles (P-ELNs) have emerged as a promising therapeutic alternative for various diseases. Our recent publication highlights the therapeutic potential of P-ELNs particularly, ginger-derived exosome-like nanoparticles (G-ELNs), in managing metabolic disorders like T2DM. The research findings demonstrated that treatment with G-ELNs improved insulin signaling, and downregulated gluconeogenesis and inflammation in insulin-resistant HepG2 cells. Furthermore, oral administration of G-ELNs in T2DM mice decreased fasting blood glucose levels and improved hepatic insulin signaling, glucose, and lipid metabolism which exerted a protective effect

on streptozotocin-induced pancreatic β -cells damage, thus contributing to systemic amelioration of T2DM. These findings revealed the potential of G-ELNs in modulating cellular responses, which prompted deeper investigations into the molecular mechanisms of their underlying effects. To elucidate these mechanisms, we shifted our attention to small RNAs particularly miRNAs (one of the crucial biocomponents) present in G-ELNs because of their known ability to modulate cellular responses via binding to target mRNAs, thereby influencing gene expression. Hence, small RNA sequencing of G-ELNs was performed which revealed the presence of 121 distinct miRNAs in them. Further, computational tools such as miRDB was employed to find out the targets of G-ELN miRNAs in human and mice genomes. KEGG annotation of these targets positively indicated their enrichment in PI3K/Akt-2 (insulin signalling) pathway. This pathway plays a crucial role in enhancing hepatic insulin sensitivity and maintaining glucose and lipid homeostasis. miRanda, a computational tool revealed that G-ELN miRNAs exhibit strong interactions with various target mRNAs, contributing to the downregulation of gluconeogenesis, ectopic lipid deposition and oxidative stress. In order to validate the computational data, synthetic mimics of G-ELNs miRNAs (2'-O-methylated mimics) were used which were found to result in effective downregulation of their target mRNAs in insulin-resistant HepG2 cells, further supporting their therapeutic potential. Overall, it was concluded that the miRNAs in G-ELNs offer a promising avenue for targeting hepatic metabolism and improving insulin signaling in T2DM

Publication:

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POSTER NUMBER: 15

THE EARLY TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL RESPONSES TO FLUCONAZOLE IN SENSITIVE AND RESISTANT CANDIDA ALBICANS

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Candida albicans is a leading cause of fungal infections in immunocompromised patients. Management of candidemia relies on a few antifungal agents, with fluconazole being first line therapy. The emergence of fluconazole-resistant strains highlights the pressing need to improve our molecular understanding of the drug response mechanisms. By sequencing the 5'P mRNA degradation intermediates, we establish that co-translational mRNA decay occurs in *C. albicans* and characterize how in vivo 5' \rightarrow 3' exonuclease degradation trails the last translating ribosome. Thus, the study of the 5' Phosphorylated mRNA degradome (5PSeq) offers a simple and affordable way to measure ribosome dynamics and identify codon specific ribosome stalls in response to drugs and amino acid deprivation. Building upon this, we combine RNA-Seq and 5PSeq to study the early response of sensitive and resistant *C. albicans* isolates to fluconazole. Our results highlight that transcriptional responses, rather than changes in ribosome dynamics, are the main driver of *Candida* resistance to fluconazole.

POSTER NUMBER: 16

Taming an endogenous retrovirus

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The horizontal transfer of transposable elements poses a significant challenge to organisms, necessitating rapid adaptation of their silencing systems to counter the new genetic threat. However, how hosts initially respond to a new invasion of transposable elements is not well understood. Here, we investigate how the Ty3/gypsy retrovirus tirant, which invaded the genome of *Drosophila melanogaster* populations within the last century, is silenced by the PIWI-interacting RNA (piRNA) pathway. By analysing *Drosophila melanogaster* strains captured around the time of the invasion of tirant, we show that a robust, de novo piRNA response against tirant is acquired through two distinct types of transposon insertions. Antisense insertions of tirant into the heterochromatic flamenco piRNA cluster represent the dominant path to immunity as previously suggested, but remarkably, single antisense insertions in the 3' UTRs of genes expressed in the gonads provide an equally potent, independent silencing mechanism. These findings highlight the critical role of antisense transposon transcripts in the cytoplasm, and not their genomic origin, in initiating piRNA-mediated silencing. This provides direct experimental evidence supporting a recently proposed model in mammals, where antisense insertions in 3' UTRs or downstream transcribed regions of host genes are central to taming invading retroviruses and highlight an unsuspected plasticity in the de novo response of hosts to new invasions of transposable elements.

POSTER NUMBER: 17

A Conserved Ribonuclease Cleaves an sRNA via a Conformational Switch: CryoEM Insights

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Bacterial small RNAs (sRNAs) play essential roles in post-transcriptional gene regulation, particularly in response to environmental stresses. In *Escherichia coli*, RyhB is a well-established model for sRNA-mediated regulation that modulates the stability and translation of over 20 mRNA targets via base-pairing. It modulates iron homeostasis enabling cells to prioritize iron allocation during scarcity by repressing non-essential iron-utilizing mRNAs (e.g., *sodB*) while promoting iron-sparing pathways. While much is known about RyhB's function, the mechanisms governing its degradation remain less understood. Here, we characterize a recently discovered ribonuclease that specifically cleaves RyhB, shedding light on bacterial RNA decay and sRNA regulation.

Through in vitro cleavage assays, we mapped the enzyme's cleavage site on RyhB and identified a recognition motif that facilitates targeted degradation. Structural analysis via cryo-electron microscopy (cryoEM) revealed a large conformational change upon RNA binding, transitioning the ribonuclease from an open inactive apo state to a closed catalytically active conformation. This structural rearrangement appears essential for substrate recognition and catalysis, distinguishing it from housekeeping ribonucleases involved in bulk RNA turnover like RNase E.

To investigate its biological role, we are conducting in vivo validation using northern blot analysis to detect

cleavage at the predicted site and assess its impact on RyhB stability under both normal and iron-limiting conditions. Additionally, mutational analyses targeting key residues—identified from apo and RNA-bound cryoEM structures—are being performed to dissect the molecular basis of this conformational change. The functional consequences of these mutations are assessed using a fluorescent reporter assay, where an mRNA target of RyhB, *sodB*, is fused to the sequence encoding a fluorescent protein. Disrupting ribonuclease activity stabilizes RyhB, reducing *sodB* repression and increasing fluorescence, providing a live-cell readout of degradation.

Comparative sequence analyses reveal that this ribonuclease is widely conserved across Gram-positive and Gram-negative bacteria, including species that lack RyhB. This conservation implies that the enzyme has additional, yet unidentified, RNA substrates, indicating a broader role in bacterial RNA regulation beyond iron metabolism.

Our findings reveal a novel RNA degradation mechanism that couples conformational changes to substrate recognition, expanding our understanding of bacterial RNA decay pathways. Since RyhB serves as a widely used model for sRNA regulation, these insights may extend to other bacterial sRNAs involved in stress adaptation. Furthermore, given its conservation in pathogens, this ribonuclease could play a role in virulence-associated regulatory circuits, making it an intriguing candidate for further study. By presenting this research, I aim to discuss its functional and structural implications with experts in the field and explore its potential impact on RNA biology, gene regulation, and biotechnological applications.

POSTER NUMBER: 18

Optical tweezers and single molecules: control, manipulate and visualize protein-RNA interactions and conformations in real-time

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Imagine you could directly see the location and dynamics of individual proteins binding to a single piece of RNA in real time. What if you could hold a RNA structural element like a hairpin, and manipulate its structure to interrogate its conformational landscape? What if you could assemble your biological complex step by step and expose it to different buffer conditions to test your experimental hypotheses?

With the LUMICKS C-Trap, the world's first dynamic single-molecule microscope combining high-resolution optical tweezers, fluorescence microscopy, and advanced microfluidics in a truly integrated system, you can do all of this! We will illustrate how the dynamic single-molecule approach can shed light on a multitude of biological processes: RNA-based DNA editing tools, translational regulation and RNA-protein condensates

These experiments show that technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument enabling control, visualization and manipulation of single molecules in real time. This gives researchers the power to directly prove molecular mechanisms, in ways not previously possible, allowing you to answer mechanistic questions faster.

POSTER NUMBER: 19

Investigating the functional divergence of Dicer's helicase domain

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The innate immune response in invertebrates such as arthropods and nematodes relies on a process known as RNA interference (RNAi). A key player in this pathway is an enzyme, Dicer, which uses its helicase domain to bind viral double-stranded RNA (dsRNA). From there, Dicer uses the energy from ATP hydrolysis to translocate and cleave dsRNAs into small-interfering RNAs. In contrast, vertebrate innate antiviral defense is dominated by a class of enzymes known as RIG-I-like receptors, which use a helicase domain related to Dicer's to bind viral dsRNA and trigger an interferon response. Dicer is conserved in vertebrates; however, its primary role is to cleave pre-microRNAs into mature microRNAs through helicase-independent mechanisms. We sought to investigate the evolutionary path leading to the loss of human Dicer's helicase activity using phylogenetics and ancestral protein reconstruction (APR). APR predicts the sequence of ancestral proteins corresponding to nodes on the phylogenetic tree based on input sequences. We generated a phylogenetic tree using extant protein sequences of animal Dicer's helicase + DUF283 domains and selected key nodes on the tree to express recombinantly and biochemically characterize. Ancestral proteins were assayed for three main helicase functions: dsRNA-dependent ATP hydrolysis, dsRNA binding, and ATP-dependent translocation. We found that the last common ancestor before the Dicer-1 and Dicer-2 gene duplication had dsRNA-dependent ATPase function, but this activity was lost entirely at the onset of vertebrate evolution. The ability of Dicer's helicase to bind dsRNA substrates declined significantly in the ancestor of bilaterian Dicer, coinciding with a decrease in ATP hydrolysis and translocation. Correspondingly, these activities were lost in both protostome and deuterostome lineages. On the other hand, ATP hydrolysis was singularly retained until the ancestor of vertebrate Dicer. Using these findings, we hope to engineer an ATP-dependent human Dicer that could participate in antiviral defense in humans. Deeper understanding of the functional incompatibility of an ATP-dependent Dicer with the interferon system in vertebrates will provide further insight into why Dicer is not involved in antiviral defense in human somatic cells and how this function could potentially be restored.

POSTER NUMBER: 20

High-throughput evolution evidence for the mechanism of the fluoride riboswitch

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Riboswitches are key structural elements typically in the 5' UTR of genes in bacteria that regulate expression in response to environmental stimuli. The fluoride riboswitch is especially impressive in that it controls transcription within 80 nucleotides. However, the sequence-structure-function relationship between ligand sensing and the mechanism of the structural rearrangement to terminate transcription is poorly understood. We initially used functional and all-atom molecular dynamic simulation methods to reveal how a single pyrimidine identity at a crucial structural junction could control the rearrangement process(1).

Here, we apply high-throughput methods to explore the sequence-structure-function relationship from a

wider lens. We acquire over 1,000 fluoride riboswitch variants and predicted which operate at the transcriptional level. We next used massive parallel oligo synthesis to generate a library of the variants and measured riboswitch function through next-generation sequencing. We observe strong correlation between predicted and measured transcription termination sites. However, we also measure twice the number of transcriptional variants than predicted. We validate the high-throughput measurements through low-throughput in vitro transcription for previously characterized variants as well as measured high-performers in our assay.

To investigate if the previously observed folding pathway for the fluoride riboswitch is conserved throughout evolution, we build a covariation model. We find several base pairs significantly covary between the two functional structures of the riboswitch. Further exploration of potential folding pathways for transcription fluoride riboswitches reveals novel pathways as hypothesized through structure predictions. Such folding pathway variability reinforces the inherent structural dynamics of RNA molecules, even those performing the same functions. This highlights the complexity of RNA structural mechanisms for consideration when building machine learning models and pursuing biotechnology developments.

(1) Hertz, L. M. et al. The effect of pseudoknot base pairing on cotranscriptional structural switching of the fluoride riboswitch. *Nucleic Acids Research*, doi:10.1093/nar/gkae231 (2024).

POSTER NUMBER: 21

Using single-molecule imaging to investigate IRE1 oligomerization during XBP1 splicing and mRNA decay

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The unfolded protein response (UPR) comprises a set of signaling pathways that monitor protein folding homeostasis in the endoplasmic reticulum (ER) and tune the protein-folding capacity of the ER through transcriptional-, and translational control, as well as mRNA decay. One of the most conserved and the best-studied branches of the UPR is activated by the ER transmembrane kinase/ribonuclease IRE1 α . Upon activation, IRE1 relays ER stress by non-canonically splicing the X-box binding protein 1 (XBP1) mRNA, which then encodes a potent transcription factor that initiates transcription of stress response genes in the nucleus. In addition to this function, IRE1 can also cleave a range of mRNAs and facilitate their rapid turnover in a process known as regulated IRE1-dependent decay (RIDD). Activation of IRE1 is associated with its dimerization and/or oligomerization, which enables trans-autophosphorylation of its cytoplasmic domains. Although previous studies have shown that ectopically expressed IRE1 assembles into large foci, there is no direct evidence that these foci are, in fact, the XBP1 processing sites on the ER. In addition, the precise oligomeric configuration adopted by IRE1 during XBP1 splicing and RIDD has not yet been elucidated. In this study, we aim to expand our previously established live single-mRNA imaging approach [1] to also allow visualization of individual IRE1 molecules in combination with different mRNA targets on the ER.

For improved detection of IRE1 oligomers at high temporal resolution in living cells, we employ a signal amplification strategy that involves a “spaghetti monster” (SM) tag, which features multiple antigen repeats on solvent-accessible loops. Co-expression of single-chain antibodies (scABs) against these repeats, in combination with the highly-photostable fluorescent proteins (e.g. mStayGOLD), enables detection and tracking of individual SM-IRE1 fusion proteins over extended periods of time.

Through successful calibration, this method will allow us to directly quantify the oligomeric state of the IRE1 assemblies that recruit/co-localize with different mRNA targets at specific time points during the UPR. Here, we present proof-of-principle experiments demonstrating that this technique allows robust detection of

SM-tagged ER membrane proteins through recruitment of scAB-mStayGOLD.

[1] Gómez-Puerta, S., Ferrero, R., Hochstoeger, T., Zubiri, I., Chao, J., Aragón, T. & Voigt, F. (2022). Live imaging of the co-translational recruitment of XBP1 mRNA to the ER and its processing by diffuse, non-polarized IRE1 α . *eLife* 11:e75580. <https://doi.org/10.7554/eLife.75580>

POSTER NUMBER: 22

Coding relationship links RNA G-quadruplexes and protein RGG motifs

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A number of RNA-binding proteins exhibit autoregulatory behavior by directly interacting with their own mRNAs. The so-called mRNA/protein complementarity hypothesis suggests that, in fact, there exists a general tendency for proteins to interact with their autogenous mRNAs, based on the idea that the genetic code has in part evolved from individual binding preferences between amino acids and their respective codons. While multiple evidence hints at this proposal on a global level, a concrete and physically realistic example of such interactions has remained elusive. Here, we focus on the relationship between a common RNA-binding domain - the arginine and glycine rich RGG motif, and a known binding partner of this domain - the RNA G-quadruplex (rG4), a four-stranded RNA structure formed by guanine-rich sequences. Importantly, arginine and glycine are encoded by guanine-rich codons, suggesting that a guanine-rich mRNA sequence could fold into an rG4, while at the same time coding for an RGG motif, potentially facilitating autogenous interactions between the two. By analyzing transcriptome-wide rG4 datasets, we show that hundreds of RGG motifs in human are at least partly encoded by rG4s, with an increased incidence for longer RGG motifs (~10 or more residues). Using randomized genetic codes, we demonstrate that the rG4/RGG coding relationship derives from the universal genetic code's structure. Moreover, we show that proteins, which contain RGG motifs encoded by experimentally detected rG4s, are significantly enriched in RNA binding relative to all RGG-containing proteins. Finally, using enhanced crosslinking and immunoprecipitation (eCLIP) data, we identify several prominent RBPs, including FUS, FMRP, and G3BP1, which interact with autogenous mRNAs in regions where RGG motifs are encoded by rG4s. Our results define a physically realistic mechanism behind autogenous mRNA/protein interactions that is hardwired in the genetic code structure and may contribute to the establishment of autoregulatory feedback loops in the cell.

References

M Adlhart, D Hoffmann, AA Polyansky & B Žagrović, *Proceedings of the National Academy of Sciences USA*, 122, 2025, <https://doi.org/10.1073/pnas.2413721122>

POSTER NUMBER: 23

From genetic code to phase separation: How composition shapes RNA-protein interactions

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RNA-binding proteins (RBP) play critical roles in cellular homeostasis and are involved in the pathogenesis of different diseases, yet the mechanism of how they interact with target RNAs remains incompletely understood. Recent transcriptome-wide studies have unveiled the large-scale structure of intracellular interaction networks involving hundreds of RBPs and thousands of mRNAs. Importantly, these networks display enormous complexity and diversity going far beyond canonical binding motifs, which creates a need for novel explanatory paradigms. Here, we describe a generalizable binding mechanism behind RNA-protein interactions at a large scale, which is embedded in fundamental physicochemical principles and places importance on the intrinsic binding affinities between individual nucleobases and amino-acids. Importantly, nucleobase/amino-acid affinities have been shown to be related to the structure of the universal genetic code and may reflect the driving forces behind its primordial origin. By analyzing a large comprehensive set of human eCLIP data, we reveal a statistically robust and significant interaction preference, wherein the amino-acid composition of RBPs reflects the amino-acid composition of proteins encoded by their target mRNAs. Furthermore, we detect similarly strong compositional parallels between mRNAs enriched in stress granules (SGs) and P-bodies and the proteins localized within these structures, pointing at an important role of intrinsic nucleobase/amino-acid affinities in guiding mRNA compartmentalization into phase separated condensates. Specifically, using a gradient boosting model, we accurately predict mRNA enrichment in SGs based solely on codon composition. Importantly, codon composition in SG-enriched mRNAs corresponds to the amino-acid composition of proteins in these phase-separated compartments, which is especially pronounced for polar and charged residues. These findings provide mechanistic insights into mRNA localization and compartmentalization, driven by compositional similarities between mRNAs and their interacting proteins. Overall, our results support a functional link between coding and binding, wherein the universal genetic code serves as a dictionary to decipher RNA-protein interactions at a large scale.

POSTER NUMBER: 24

From PIWI to RdRP: Functional Divergence of GTSF-1 in Nematodes

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GTSF-1 is a highly conserved Zinc-finger protein that associates with PIWI Argonaute and is crucial for piRNA-directed silencing in mice, flies and insects. GTSF-1 is deeply conserved in animals, including the nematode *C. elegans*. *C. elegans* have piRNAs associated with PIWI protein PRG-1, targeting transposable elements and regulating fertility. However, *C. elegans* GTSF-1 is shown to associate with an RNA-Dependent

RNA Polymerase (RdRP) named RRF-3, deviating from the conventional PIWI association (Almeida et al., 2018). The observation gains significance as several nematode lineages independently lost the entire piRNA pathway, yet retained RRF-3-like RdRPs (Sarkies et al., 2015). This study seeks to understand the factors conferring specificity of *C. elegans* GTSF-1 to RRF-3 and investigate the conservation of this association across nematodes.

With IP-MS, we describe a robust and specific interaction between GTSF-1 and RRF-3 in *C. briggsae* and *P. pacificus*. In both species, deletion of *gtsf-1* resulted in sterility at elevated temperatures and a specific loss of 26G-RNAs (endo-siRNAs), while 21U-RNAs (piRNAs) remained unaffected. We predicted the interactions of nematode GTSF-1 with RRF-3 using AlphaFold 2/3 and validated these interactions through yeast-two-hybrid assays and in-vivo mutations. We show the NTD of GTSF-1 is sufficient to bind RRF-3 and propose functions for the CTD in bridging RRF-3 to its functional partners. Furthermore, we describe a novel GTSF-1 interacting domain (GID) in the N-terminus of RRF-3. Finally, we identified key residues in both proteins that mediate this interaction. Our results suggest that the GID may contribute to the specificity of GTSF-1 for RRF-3, as other *C. elegans* RdRPs possess a structurally similar domain but lack the residues required for GTSF-1 interaction.

We also describe sequence and structure differences in nematode GTSF-1 that may explain loss of PIWI binding. The central region lacks aromatic residues, crucial for PIWI binding in mice and insects. Additionally, *C. elegans* GTSF-1 does not bind RNA in-vitro, likely due to the absence of a positively charged region near the N-terminal Zn-fingers (Ipsaro et al., 2021). Notably, AlphaFold predicts strong interactions between *M. musculus* and *B. mori* GTSF-1 with PRG-1, whereas *C. elegans* GTSF-1 shows poor binding scores with both PRG-1 and *M. musculus* PIWI.

Our results from reciprocal BLASTp finds GTSF-1 to be conserved across the phylum nematoda- paralleling RRF-3 rather than PIWI. As GTSF-1 supports PIWI function even in ciliates (Charmant et al., 2024), our work places nematoda as the only known system where GTSF-1 evolved to bind an RdRP. We are further investigating the origins of this divergence by using AlphaFold to screen GTSF-1, PIWI, and RRF-3 interactions across additional species, as well as test conservation of the GID across RRF-3 homologs. We hypothesize that despite differences in binding partners, GTSF proteins may share a conserved role in inducing conformational changes in either PIWI or RdRP to support their function. Finally, we discuss how this unique adaptation may explain the repeated loss of the piRNA pathway across nematode lineages.

POSTER NUMBER: 25

Competition between pre-mRNA stabilization and decay pathways regulates mitochondrial function in development and disease

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The nuclear cap-binding complex (CBC), which consists of CBP20 and CBP80, binds the 5'-end cap of nuclear RNAs synthesized by RNAPII. The CBC has been implicated in numerous RNA quality and quantity control mechanisms that regulate gene expression in the nucleus [1] and in the cytoplasm [2]. For example, we recently reported that the transcriptional co-activator PGC-1 α senses CBP80 at the cap of nascent pre-mRNAs deriving from stress-activated genes during promoter-proximal pausing of RNAPII to allow for transcription elongation [3,4]. We used an in-house mouse model to show that this mechanism ensures timely skeletal-muscle regeneration after injury.

Following release of RNAPII from pausing, the CBC recruits ARS2 to form the CBCA, a sorting platform for nuclear RNAs [1]. However, how the CBCA determines the fate of pre-mRNAs remains largely unknown [5]. We report that the CBCA-binding protein NCBP3 [6] activates specific genes in skeletal muscle cells by stabilizing chromatin-associated intron-containing polyadenylated pre-mRNAs that we call PIA-chrRNAs. Mechanistically, NCBP3 competes with the scaffold protein Anillin at the CBCA to determine the fate of PIA-chrRNAs. While NCBP3 is required for the stabilization PIA-chrRNAs, thereby allowing their post-transcriptional splicing to mRNAs and nuclear export, Anillin triggers PIA-chrRNA decay by the nuclear RNA exosome.

Most NCBP3-stabilized PIA-chrRNAs encode mitochondrial electron transport chain factors with strong developmental and disease relevance. For example, PIA-chrRNA metabolism is regulated during myogenesis, i.e. the multi-day differentiation of skeletal muscle cells, a developmental process heavily relying on mitochondrial biology. First, NCBP3 and PIA-chrRNAs accumulate during early myogenesis, when myoblasts commit to differentiation. Second, NCBP3 is downregulated and PIA-chrRNAs are spliced during late myogenesis, when myoblasts fuse to form myotubes. Additionally, mutations in RNA exosome subunits result in upregulation of NCBP3-stabilized PIA-chrRNAs, mitochondrial dysfunction, and the development complex neuromuscular disorders. Ncbp3-KO mice also exhibit muscular and neurodevelopment defects. Our findings reveal a previously unanticipated pre-mRNA processing pathway whose balance controls mitochondrial biology that, when perturbed, leads to disease.

1 Rambout X & Maquat LE. The nuclear cap-binding complex as choreographer of gene transcription and pre-mRNA processing. *Genes & Development* 34, 1113-1127 (2020).

2 Kurosaki T, Rambout X & Maquat LE. FMRP-mediated spatial regulation of physiologic NMD targets in neuronal cells. *Genome Biol* 25, 31 (2024).

3 Cho H, Rambout X et al. Transcriptional coactivator PGC-1 α contains a novel CBP80-binding motif that

orchestrates efficient target gene expression. *Genes Dev* 32, 555-567 (2018).

4 Rambout X. et al. PGC-1 α senses the CBC of pre-mRNA to dictate the fate of promoter-proximally paused RNAPII. *Mol Cell* 83, 186-202.e111 (2023).

5 Rambout X & Maquat LE. Nuclear mRNA decay: regulatory networks that control gene expression. *Nat Rev Genet* (2024).

6 Rambout X & Maquat LE. NCBP3: A multifaceted adaptive regulator of gene expression. *Trends Biochem Sci* 46, 87-96 (2021).

POSTER NUMBER: 26

Uncovering the Secrets of RNA Localization in Female Infertility

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While once dismissed as “transcriptional noise,” non-coding RNAs (ncRNAs) are now recognized as critical regulators of cellular processes. This is particularly relevant in mammalian oocytes, where ncRNAs are abundantly expressed and implicated in meiotic maturation, embryonic development, and follicular development. We previously investigated the expression, localization, and potential functions of several ncRNAs during meiotic maturation and early embryo development in mice. We analyzed Brain cytoplasmic RNA 1 (BC1), lncRNA in Oocyte Specifically Expressed (Rose), RNA Component of 7SK Nuclear Ribonucleoprotein (Rn7sk), Nuclear Enriched Abundant Transcript 2 (Neat2), Intracisternal A Particle Long terminal repeat RNA (IAPLtr1a) and snoRNA. Our findings reveal distinct nuclear or cytoplasmic localization patterns for these ncRNAs at the germinal vesicle (GV) stage, with declining expression during maturation and development. Additionally, we examined ncRNA localization in a mouse model with CDK12 conditional knockout in oocytes, which exhibits a premature ovarian failure (POF) phenotype. Our results provide insights into the involvement of ncRNAs in oocyte development and their potential contribution to POF pathology. The elucidation of these RNA regulatory networks generates knowledge that could therefore be used to improve our diagnostic capabilities for human infertility and treatment strategies.

POSTER NUMBER: 27

Deciphering the Molecular Mechanism underlying Cluster Assistance in miRNA Biogenesis

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MicroRNAs (miRNAs) are a species of small non-coding RNAs that guide the RNA-induced silencing complex (RISC) to complementary sites in mRNAs, creating a layer of post-transcriptional regulation of protein expression. Mature miRNAs are produced in a multistep process, initiated by the recognition and cleavage of hairpin-shaped folds in long primary RNA transcripts by the Microprocessor, a protein complex composed of the RNase DROSHA and the RNA binding protein DGCR8. Several structural features and sequence motifs

in and around the primary miRNA hairpin determine whether it is recognized as a substrate by the Microprocessor, and how efficiently it is cleaved. In this manner, the Microprocessor functions as a gatekeeper, deciding which of all of the many hairpin-shaped folds found across the whole transcriptome actually enter the miRNA biogenesis pathway. However, the Microprocessor's normally stringent substrate selectivity can relax substantially in a phenomenon termed "cluster assistance", in which an optimal hairpin can enhance or even enable the processing of a suboptimal one, if both are located close to each other on the same primary transcript, i.e. clustered and in cis.

We currently lack a concrete understanding of the molecular mechanisms behind this peculiar phenomenon. Cluster assistance depends on at least two accessory factors, SAFB2 and ERH, and both interact with the Microprocessor and with each other. I plan to discuss our current understanding of the distinct roles of both factors in cluster assistance and present our working hypothesis of how these proteins may alter the Microprocessor's substrate selectivity. Furthermore, we have uncovered that the structurally disordered N-terminus of DGCR8 plays a critical role in cluster assistance and we have been able to pinpoint two evolutionarily conserved sequence motifs within it, which are not only essential, but even sufficient to drive the process. Thus, our data describe a novel role for the previously uncharacterized DGCR8 N-terminus in miRNA biogenesis.

POSTER NUMBER: 28

Modulation of miRNA Biogenesis by Ionizing Radiation in Burkitt Lymphoma Cells

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Ionizing radiation (IR) is a key component of cancer therapy, inducing cell death through DNA damage and triggering cellular stress responses. MicroRNAs (miRNAs) contribute to the radiosensitivity of cancer cells. miRNA biogenesis involve processing of primary miRNAs (pri-miRNAs) into precursor miRNAs (pre-miRNAs), followed by cytoplasmic maturation to single-stranded miRNA.

In this study, we examined the effect of ionizing radiation on miRNA biogenesis in three Burkitt's lymphoma (BL) cell lines, i.e. CA46, DG75 and ST486. All 3 cell lines induced apoptosis and cell cycle arrest in the G2/M phase after exposure to 4Gy of IR. To investigate changes in miRNA processing, we performed RNA sequencing and small RNA sequencing at 4 and 12 hours after irradiation, comparing miRNA and pri-miRNA levels. Pri-miRNA was defined as pre-miRNA plus 150 nucleotides of flanking sequences. We identified 189 miRNAs and 429 pri-miRNAs consistently expressed in three BL cell lines. We found that IR affected the levels of a limited subset of the miRNAs, with miR-146a, miR-449c and miR-449a showing at least a 1.5-fold increase in three and miR-155 in two BL cell lines after IR. Next, we identified miRNAs with discrepancies in the miRNA to pri-miRNA ratios upon IR that may indicate regulated miRNA processing. Interestingly, we also identified 186 pri-miRNAs that were not processed to mature miRNAs in any of the 3 cell lines. To further dissect miRNA processing kinetics, we quantified levels of pri-miRNA and mature miR-146a, miR-155, and miR-449a by qRT-PCR at 1, 4, 8, 12, and 24 hours post IR. Pri-miR-146a and pri-miR-155 levels increased within 1–4 hours post IR, preceding the increase in mature miRNA levels, suggesting IR-induced transcriptional activation.

miR-449a displayed a rapid processing pattern, with undetectable pri-miRNA levels but peak mature miRNA expression at 8 hours. In conclusion, our findings showed that IR modulated the biogenesis miRNAs in BL cells, in particular miR-155, miR-146a and miR-449a.

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POSTER NUMBER: 29

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

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

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

POSTER NUMBER: 30

Nascent RNA Decay via the Nuclear Exosome is Essential for Piwi-Mediated Transposon Silencing

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Transcriptional gene silencing via small RNA pathways has been studied primarily in the context of heterochromatin formation, while the molecular mechanisms acting directly on nascent target RNAs remain understudied. Here, we reveal an essential role of the nuclear RNA exosome in Piwi-piRNA-mediated transposon repression. Our findings demonstrate that the three nuclear RNA exosome adaptor complexes—PAXT, TRAMP, and NEXT—act redundantly to specify the degradation of piRNA target transcripts. Through comprehensive profiling of the proteome at piRNA targets, we identified two uncharacterized piRNA pathway factors, Tesup-1 and Tesup-2, which link the RNA exosome to the Piwi-silencing complex via a unique polyproline-binding domain. These findings uncover a critical silencing mechanism in the nuclear piRNA pathway that controls nascent target RNA fate, expanding our understanding of small RNA-guided gene silencing beyond heterochromatin formation.

POSTER NUMBER: 31

Investigating mRNA targeting mechanisms and functional relevance of Dync1h1 granules in mouse intestinal organoids using single-molecule imaging.

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In recent years, the gut epithelium has emerged as a powerful system to study mRNA localization. It consists of a single-cell layer with specialized functions on the apical and basal sides, resulting in a well-defined apicobasal polarization, with mRNA localization biases along this axis. This asymmetrical distribution is maintained due to the cytoskeletal organization in epithelial cells, where motor proteins (dynein and kinesin) transport cargo along non-centrosomal microtubules arranged from apical to basal ends. Dynein transports cargo toward the microtubule minus ends located on the apical side, while kinesins transport cargo to the plus ends on the basal side.

Interestingly, the mRNA encoding the heavy chain of the cytoplasmic dynein complex, Dync1h1, has been

reported to form translation-dependent granules in multiple cell lines. Moreover, it was observed that in two different epithelial tissues these granules display an extra layer of regulation, as they consistently accumulate on the apical side. What are the mechanisms behind this apical accumulation? Are they anchored to a particular cellular structure? Or are they so big that diffusion is extremely slow? What is the physiological relevance of these granules and their apical localization? Could the accumulation of multiple subunits favor co-translational complex assembly?

Here, we show that murine intestinal organoids recapitulate these localization patterns, allowing us to study their targeting mechanisms and functional implications. The results of our perturbation experiments with translation inhibitors (puromycin, cycloheximide) indicate that granule formation and apical localization are mediated by either nascent chain or translation machinery interactions. Moreover, preliminary evidence from single-molecule FISH (smFISH) against other cytoplasmic dynein subunits and cargo adaptors shows heterogeneity in transcript localization, with mRNAs from certain cargo adaptors consistently localizing close to—but not together with—Dync1h1 granules.

Future experiments aim to dissect the localization mechanisms and functional relevance of these granules, and to increase the throughput of our smFISH assay by performing sequential rounds of probe hybridization and imaging using an automated microfluidics and image acquisition set-up. This will allow us to investigate the localization patterns and perform colocalization analysis of multiple dynein-related transcripts. Additionally, by using mouse intestinal organoids, we will be able to study these patterns across different cell types and under multiple perturbations (e.g., translation inhibition, microtubule depolymerization, dynein inhibition, starvation, etc). Lastly, to further study targeting mechanisms and translation efficiency we aim to characterize the granule recruitment/anchoring and translation dynamics using live single-molecule imaging.

POSTER NUMBER: 32

sRNA biogenesis in duckweed: the transitivity dilemma

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Exogenous gene silencing is crucial to plants as a defense mechanism against virus and unwanted pathogens, mainly by using siRNAs. Transitivity amplifies the response of silencing by producing more of these siRNAs to target different regions of the foreign transcripts or transposons. However, this response is not found in duckweeds. Why is it that this happens? In this research we aim to understand the mechanism behind the lack of transitivity in duckweed.

POSTER NUMBER: 33

A comprehensive interrogation of transcription factors in mouse embryonic stem cells

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Mammalian genomes encode over 1,500 putative DNA-binding proteins, collectively known as transcription factors (TFs), to designate genes for coordinated expression. Despite the critical role of TFs in cell biology, the genomic locations of their binding sites and their interaction partners remain largely enigmatic. This is due to the limitations of state-of-the-art methods, which rely on the availability of specific, validated antibodies—a resource that is often scarce. To address this challenge, the Bühler lab has endogenously tagged all TFs (~ 800) expressed in mouse embryonic stem cells (mESCs) with the same V5-3xFLAG epitope. Leveraging this collection of cell lines, we are systematically mapping the interactomes and genomic binding sites of hundreds of TFs. This resource can be capitalised for functional interrogation and further characterisation of TFs. Here, I propose a strategy to utilize a degron fused to a nanobody that binds the V5 tag in the cell line collection of the Bühler lab. Depletion of any given TF in the collection is conceivable by this method for unveiling primary targets and dose-dependent action of TFs. The emergence of de novo protein design algorithms expands our repertoire of functionalised binders to allow the mapping of all protein and RNA interactors of a given TF with minimised background. Together, this work harnesses protein engineering to investigate unresolved problems in gene regulation at a massive scale.

POSTER NUMBER: 34

Germline small RNA modification in epigenetic inheritance

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In eukaryotes, epigenetic reprogramming takes place during gametogenesis to restore pluripotency and reset developmental programs. Plants are an exception, as some epigenetic modifications can be inherited despite reprogramming. Although epigenetically activated small interfering RNAs (easiRNA) are known to mediate the deposition of epigenetic marks at imprinted genes, their inheritance through gametogenesis and later fertilization remains poorly understood. Recently, easiRNAs with pseudouridine modifications were shown to be required for establishing hybridization barriers known as the triploid block in *Arabidopsis thaliana*, and easiRNA pseudouridylation was suggested to be key for their inheritance. We propose a molecular and computational approach to investigate pseudouridylated-easiRNA inheritance and their role in the establishment of the triploid block. To determine the function of pseudouridine for the inheritance of easiRNAs, we aim to develop a system for targeted easiRNA pseudouridylation in pollen combined with small RNA sequencing of the endosperm. Using a CRISPR-guided RNA editing approach, we intend to artificially induce pseudouridylation of selected microRNA, and to investigate whether this artificial pseudouridylation affects

their mobility and, subsequently, their inheritance in the male germline. Additionally, we aim to explore whether hairpin-originated pseudouridylated-easiRNA and their associated targets in *Arabidopsis thaliana* establish reproductive barriers in a similar way to the Teosinte Pollen Drive system described in Maize. By investigating pseudouridylated easiRNA targets, we hope to also decipher their role in genomic imprinting. Understanding inheritance of easiRNAs in the establishment of hybridization barriers will provide a better understanding of plant evolution and domestication, and contribute to the development of new plant breeding tools to overcome hybrid incompatibilities in modern crops.

POSTER NUMBER: 35

SARS-CoV-2 Nsp1 interacts with stalled ribosomes and promotes stall read-through

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, intervenes in the host's translational machinery during infection. This phenomenon is mediated chiefly by non-structural protein 1 (nsp1), a viral protein known to suppress translational initiation of host transcripts by binding the 40S ribosomal subunit on the mRNA entry channel. Early structural and biochemical studies suggest the occlusion of the mRNA entry channel by its C-terminal domain (CTD) sterically blocks mRNA loading to 43S pre-initiation complexes and, likewise, prevents nsp1 from interacting with mRNA-loaded 80S ribosomes. However, subsequent works suggest the role of nsp1 in promoting translation termination and suppressing the formation of translational stall-mediated truncated proteins, throwing this notion into question.

Here we show that, in stark contrast of the currently accepted model, SARS-CoV-2 nsp1 binds stalled elongating 80S ribosomes and promotes their stall read-through. Through fluorescence polysome profiling and cryo-electron microscopy with purified stalled 80S complex, we demonstrate that the CTD of nsp1 mediates its interaction with the mRNA entry channel of the 40S subunit of a ribosome with an active decoding centre. Furthermore, this interaction does not affect the global translation elongation rate while, contrary to a previous report, doubling translational read-through rate of stalled ribosomes. These results demonstrate the role of nsp1 in manipulating host translation beyond merely suppressing the translation initiation of host mRNA, implying a more holistic influence to ensure the smooth expression of viral and critical host proteins in support of viral proliferation. Considering the strong structural and mechanistic conservation of nsp1 between sarbecoviruses and its importance for viral pathogenicity, our results highlight a potential novel effective target for the development of new therapeutics against COVID-19 and future coronavirus diseases.

POSTER NUMBER: 36

Upstream cues regulating Alternative splicing (AS) of auxin-dependent processes

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The phytohormone auxin plays a central role in governing nearly all facets of plant growth and development. The directional transport of auxin is crucial for ensuring explicit plant growth and development. PIN-FORMED (PIN) auxin efflux carriers are among the key regulators which form the auxin gradients and maintain polar auxin transport in plants. In parallel, alternative splicing (AS) affects numerous gene expression, resulting in the generation of potentially distinct functional protein isoforms.

AS of the PIN7 gene leads to two different transcripts. Our group has demonstrated the molecular model of the coordinated role of protein isoforms PIN7a and PIN7b arising through AS of the Arabidopsis thaliana PIN7 gene (Kashkan et al., 2021). In a follow-up project, we are seeking for the upstream regulatory mechanisms controlling this AS event. We analyzed the available transcriptomic datasets whether some of the earlier characterized RNA factors have an impact on the expression of the individual PIN7 variants. Surprisingly, our search revealed two groups of mutants in which AS of the PIN7 gene is changed: It highlights the factors participating on methylation of mRNA and a specific group of spliceosomal components. In addition, we have developed a genetic screening system with the aim of uncovering upstream factors modulating PIN7a/b splicing. With this, we aim to bring new insights into the vast regulatory potential of AS.

Reference-

Kashkan I, Hrtyan M, Retzer K, Humpolíčková J, Jayasree A, Filepová R, Vondráková Z, Simon S, Rombaut D, Jacobs TB, Frilander MJ, Hejátko J, Friml J, Petrášek J, Růžička K. Mutually opposing activity of PIN7 splicing isoforms is required for auxin-mediated tropic responses in Arabidopsis thaliana. *New Phytol.* 2022 Jan;233(1):329-343. doi: 10.1111/nph.17792.

POSTER NUMBER: 37

The edited isoform of the Drosophila Adar RNA editing enzyme regulates ecdysone signalling and innate immunity during development

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ADAR RNA editing enzymes deaminate selected adenosine bases to inosines in dsRNA regions in cellular RNAs. In Drosophila, Adar RNA pre-mRNA editing in exons gives rise to edited isoforms of very many proteins, particularly in the adult CNS. Embryos and larvae have much lower levels of Adar protein and RNA editing, which increase in response to ecdysone at metamorphosis. In flies, RNA editing in the Adar transcript generates an adult-specific Adar G isoform, which was suggested to be the isoform that inhibits RNAi and heterochromatin silencing. We show that early, ubiquitous overexpression of the adult, edited Adar G isoform leads to larval and pupal lethality, providing a screen for suppressors of Adar G overexpression defects. Surviving Act5Cts >Adar G pupae fail to eclose and show head eversion defects characteristic of excessive ecdysone signaling; a genetic screen identified RNAi knockdown of EcR A or Nos as suppressors of 50 per cent for both larval lethality and pupal defects with only females reaching the adult stage. RNA sequencing of pre-mRNA pupae also revealed high level, aberrant innate immune induction of antimicrobial peptide (AMP) transcripts and ecdysone induced transcripts. This effect is also observed in Act5Cts >AdarE374A flies expressing catalytically inactive Adar E374A protein is due to an RNA editing-independent effect of Adar protein, such binding to dsRNA and/or other proteins. The surviving adults after the knockdown of EcR-A have AMP levels similar to WT, suggesting that aberrant AMP induction may be a consequence of aberrant ecdysone responses. Furthermore, we found that Adar is normally expressed in the larval prothoracic gland (PG) cells in the ring gland that synthesize ecdysone and we identify Adar RNA editing sites in PG-specific transcripts in published ring gland transcriptomes. Overexpression of Adar G in phm22>AdarG larval PGs under Phantom-GAL4 driver control leads to large larvae which can pupate and give large adults. RNA sequencing shows severe decreases in transcripts of the spookier and neverland genes located in centric heterochromatin, which encode ecdysone synthesis enzymes. Larval lethality and pupal defects are partially rescued by adding ecdysone to the food.

POSTER NUMBER: 38

Elucidating the role of nuclear compartmentalization in testis-specific gene regulation

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Animal germline development and fertility rely on paralogs of general transcription factors to ensure cell type-specific gene expression during gametogenesis. Drosophila tPAF is a testis-specific paralog of the canonical transcription factor complex Paf1C and each tPAF subunit is essential for male fertility in Drosophila melanogaster. How transcription factor paralogs like tPAF enable animal germline development remains poorly understood.

Notably, tPAF is enriched in spermatocyte nucleoli, and we have found that this nucleolar localization depends on the tPAF subunit Cdc73L. I aim to elucidate tPAF-mediated germline development and the function of nucleolar accumulation of testis-specific gene regulatory factors. To this end, we have found that Cdc73L seems to function as a general nucleolar anchor for many RNA polymerase II transcription factors in spermatocytes. Moreover, we have identified two parts of the Cdc73L protein that confer nucleolar localization. Our research aims to uncover whether nucleolar accumulation of testis-specific gene regulatory factors supports robust transcription or relates to other processes such as genome organization.

POSTER NUMBER: 39

Regulation of protein abundance in neurons by selective translation of 3'UTR isoforms

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Precise regulation of protein levels is essential for cellular function, particularly in neurons, where dysregulated mRNA translation is linked to impaired memory formation and is a hallmark of neurodegenerative diseases. Translation efficiency is influenced by transcript features, including mRNA and 3' untranslated regions (3'UTRs) length. Longer 3'UTRs present an intriguing avenue for translational regulation, as they can extend up to tens of kilobases in length, providing a substantial platform for secondary structure formation, as well as binding sites for RNA-binding proteins (RBPs) and miRNAs. ELAV, a master regulator of alternative polyadenylation (APA) in neurons, plays a central role in this process by generating extended neuronal 3'UTRs (nUTRs) for nearly 300 genes, including *elav* itself, in *Drosophila melanogaster*. Given the prevalence of extended 3'UTRs in neuronal mRNAs, we investigated their role in translational control.

In this study, we demonstrate that mRNA isoforms with shorter 3'UTRs are more efficiently translated than their longer counterparts in *Drosophila* neurons. Specifically, we show that an *elav* mRNA isoform containing an nUTR is translated less efficiently, thereby acting as a buffering mechanism to regulate overall ELAV protein levels. This phenomenon appears to be widespread, as ELAV target genes undergoing APA similarly produce shorter isoforms with enhanced translation efficiency. Additionally, we reveal that the translation of longer isoforms is repressed by Pumilio, another RNA-binding protein involved in post-transcriptional regulation. Finally, we establish that proper ELAV protein regulation is essential for stress resilience in *Drosophila*, highlighting the physiological significance of nUTR-mediated translational control.

POSTER NUMBER: 40

Developing models to visualize translational heterogeneity and RNA localization biases in live intestinal epithelium

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RNA localization is a key mechanism of post-transcriptional gene regulation across diverse organisms and tissues. One such regulatory outcome is the modulation of translation efficiency (TE), the protein output per transcript. A notable example is the murine intestinal epithelium, where ribosome abundance and occupancy is reportedly higher in apical than basal regions (Moor et al., 2017). Thus, it is appealing to speculate how cells might fine-tune gene expression post-transcriptionally, by altering transcript localization along this TE axis. Indeed, such modulation in response to nutrient availability has been observed for transcripts encoding ribosomal proteins (RP). However, previous studies capture only static snapshots of ribosome abundance

and occupancy, overlooking dynamic processes such as initiation, elongation rates, and mRNA diffusion. Additionally, mechanisms underlying the localization and associated TE changes remain largely unknown.

To resolve differences in TE across the apical-basal axis, we are developing model systems to map the translational landscape across the gut epithelium. Specifically, we are implementing a real-time SUN-tag imaging-based approach in murine intestinal organoids. In our system, an inducible non-fluorescent GFP core, called spaghetti monster, is used to solubilize and present epitopes, which are bound by fluorescent single chain antibodies during translation. We are currently optimizing both the generation of stable organoid lines and the imaging setup to assess translation dynamics at high spatiotemporal resolution in 3D. Future work will include membrane tethers – and thus the sites of their translation – to either the apical or basal side of the cell, allowing us to refine our understanding of location specific translation dynamics.

To investigate the extent and mechanisms of nutrient-dependent RP transcript localization, we are exploring a 2D cell culture model (C2bbe1 cells), classic intestinal organoids, as well as more sophisticated organoid surface and perfusion chip models. These systems offer complementary strengths in complexity, physiological relevance, and throughput, making them suitable for distinct experimental needs. Namely, we are using smFISH in combination with different external stimuli and perturbations to better understand what drives changes in localization and TE. Additionally, we are developing a high-throughput screen to identify cis-acting sequence motifs that regulate transcript localization.

Together, these approaches aim to reveal how nutrient signals shape spatially resolved protein synthesis, offering insight into the dynamic translational control in epithelial cells.

POSTER NUMBER: 41

Investigating the molecular mechanisms underlying IRE1-dependent mRNA turnover on the ER

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Cells respond to stress in the endoplasmic reticulum (ER) by initiating the widely conserved unfolded protein response (UPR), in which the ER transmembrane nuclease IRE1 α plays a pivotal role. Upon activation under stress conditions, IRE1 degrades a subset of mRNAs to help restore ER homeostasis. These include the canonical IRE1 target X-box binding protein 1 (XBP1), as well as transcripts degraded via regulated IRE1-dependent decay (RIDD or RIDDLE). While the investigation of IRE1's role in splicing XBP1 mRNA has been extensively studied, the molecular mechanisms and underlying principles of the RIDD and RIDDLE pathways remain poorly understood. Within the lab, we recently established a live imaging approach to visualize the recruitment and colocalization of XBP1 mRNA on the ER. Building on this, I will take advantage of the established methodology to investigate the dynamics and turnover of other IRE1 targets upon induction of ER stress. To achieve this, I will first perform a time-course analysis to define the precise stress conditions that initiate the RIDD and RIDDLE pathways using distinct agents, including both specific and nonspecific ER stress inducers. Thereafter, I will use a live imaging approach to track individual reporter transcripts and their co-localization with the ER. I will verify their functionality using qPCR, smFISH and western blot analysis. Moreover, I will investigate how these cleavage pathways influence the cellular ability to recover from stress by performing transcript-specific functional analyses, e.g. looking at triglyceride and cholesterol metabolism, apoptosis, autophagy, and DNA repair.

POSTER NUMBER: 42

ALBA proteins facilitate cytoplasmic YTHDF-mediated reading of m6A in Arabidopsis

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N6-methyladenosine (m6A) exerts many of its regulatory effects on eukaryotic mRNAs by recruiting cytoplasmic YTHDF proteins. Here, we show that in *Arabidopsis thaliana*, the interaction between m6A and the major YTHDF protein ECT2 also involves the mRNA-binding ALBA protein family. ALBA and YTHDF proteins physically associate via a deeply conserved short linear motif in the intrinsically disordered region of YTHDF proteins and their mRNA target sets overlap, with ALBA4 binding sites being juxtaposed to m6A sites. These binding sites correspond to pyrimidine-rich elements previously found to be important for m6A binding to ECT2. Accordingly, both the biological functions of ECT2, and its binding to m6A targets *in vivo*, require ALBA association. Our results introduce the YTHDF-ALBA complex as the functional cytoplasmic m6A-reader in *Arabidopsis*, and define a molecular foundation for the concept of facilitated m6A reading, which increases the potential for combinatorial control of biological m6A effects.

POSTER NUMBER: 43

Histone methyltransferase SETD2 regulates transcription termination by suppressing cryptic initiation and enhancing 3' pre-mRNA cleavage

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SETD2 is a key histone methyltransferase responsible for depositing histone H3 lysine 36 trimethylation (H3K36me3). Loss of its enzymatic activity has been implicated in various cancers, including clear cell renal cell carcinoma (ccRCC). In ccRCC, SETD2 mutations have been linked to defects in transcription termination, though the underlying mechanism remains unclear.

Here, using nascent transcriptomics in ccRCC patient-derived cells and SETD2 knockouts, we show that SETD2 loss leads to delayed transcription termination, but only in a subset of genes. POINT-seq analysis reveals widespread transcriptional readthrough beyond canonical termination sites upon SETD2 depletion, affecting 15–24% of genes. This readthrough may result in transcriptional interference, disrupting the expression of downstream genes.

Mechanistically, we demonstrate that defective termination following SETD2 loss is driven by a combination of increased cryptic transcription initiation and impaired 3' pre-mRNA cleavage. In contrast, genes that maintain proper termination show significant downregulation. Additionally, we find that SETD2 influences alternative polyadenylation independently of transcriptional readthrough, underscoring the distinction

between transcription termination and 3' end processing.

Our findings highlight SETD2 as a crucial regulator of transcriptional integrity, preventing cryptic initiation and promoting efficient 3' end processing.

POSTER NUMBER: 44

Investigation the role of mitochondria in the processes of differentiation of human mesenchymal stem/progenitor cells, mouse embryonic 3T3 fibroblasts and mouse embryonic stem cells

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Three sub-populations of mouse embryonic stem cells (mESCs) were derived: transfected and containing additionally-inserted copy of oncogene *Dcn1*; transfected and containing additionally-inserted copy of tumor-suppressor gene *HACE-1*, as well as non-transfected and non-containing additionally-inserted genes mESCs. Differentiation of derived from *in vitro*-incubated adult human mesenchymal stem cells (MSCs), isolated from bone marrow, cartilage and adipose tissue, respectively, but also of mouse embryonic fibroblasts from 3T3 line in appropriate incubation conditions, to chondrocyte, osteoblast and osteoclast directions, was investigated and proven. Besides of the applied incubation conditions, genetic and/or epigenetic influences, a key role of the mitochondria in the process of cell differentiation was proposed. According to the results obtained, probably changes in the mitochondrial dynamics underlined the respective differentiation directions of all types of the used and tested low differentiated cells. In this way, the current results are in agreement with the literature data and confirmed the key role of these organelles in the differentiation direction of stem/progenitor cells. Furthermore, a possibility for development of new mitochondria-based strategies about the needs of the transplantation practice was suggested. In this relation, techniques about internal (genetic), epigenetic and external influence on the CRISPR/Cas mechanisms, should be developed. These are universal protective pathways, responsible about normal/non-malignant cell differentiation, support of adequate immune response, as well as prevention of malignant cell transformation on the one hand, but also cellular ageing and death on the other, should be influenced. So, *in vitro*-over-expression of specific genes, related with CRISPR/Cas systems activation, by transfection of laboratory-incubated stem/progenitor cells as MSCs, 3T3 mouse embryonic fibroblasts and mESCs by appropriate siRNAs against appropriate target genes should be performed. Furthermore, the established features suggest a possibility about generation of mixed cultures of the described types of low differentiated cells and further derivation of organoid and embryoid (female XX and male XY) structures, possessing different types of cells and tissues.

POSTER NUMBER: 45

COLD-INDUCED PHASE SHIFT OF MIR157 EXPRESSION MODULATES FLOWERING AND ROOT ARCHITECTURE IN ARABIDOPSIS

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Plants sense and respond to environmental cues during 24h fluctuations in their environment. This process involves integrating internal signals, like circadian rhythms, with external cues such as light and temperature to trigger cellular responses through signal transduction. SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SPL) transcription factors regulate key developmental processes, including lateral root emergence and age-dependent flowering. In *Arabidopsis thaliana*, miR156/157 are generated from eight different precursors and directly regulate 10 SPL genes. In this work, we explored the contribution of the miR156/157-SPL module in adaptation to changes in ambient temperature. We found that a member of the miR156/157 family shows a rhythmic expression pattern, peaking in the morning. Interestingly, at 22°C, only one of the the SPL target genes has a complementary expression pattern to that of the miR156/157, showing also rhythm expression but with its maximum at night. However, cold treatment induces in MIR157 a phase shift towards the end of the night period leading to the loss of rhythmic SPL expression. Both SPL mRNA and protein levels are reduced by cold in a miR157-dependent manner. Cold delays flowering and inhibits lateral root development in wild-type plants, but mutant plants for MIR157 and SPL genes are insensitive to cold regulation. Our results suggest that specific members of the MIR157/SPL family are responsible for growth reprogramming under changing temperatures.

POSTER NUMBER: 46

Potential role of AAA+ proteases in ribosome turnover

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When ribosome levels exceed cellular demands for protein biogenesis, excess particles can be targeted for recycling. During conditions that lead to slow growth (such as starvation, antibiotic treatment) ribosome degradation increases dramatically, releasing metabolites for biosynthetic pathways and ensuring that a limited number of translation events continue to completion. The antibiotic rifampicin can trigger strong ribosome degradation response, by inhibiting transcription initiation, which in turn leads to the accumulation of free ribosomes and ribosomal subunits. Ribosome turnover includes both RNA and protein turnover, and we observe concomitant turnover of ribosomal proteins along with ribosomal RNA upon rifampicin stress. Most intracellular proteolysis is initiated by the AAA+ proteases, but we observe that no single member of that protease group is solely responsible for initiating ribosome degradation. Our data suggest redundancy in contributions of proteases to ribosome catabolism.

POSTER NUMBER: 47

Investigating conserved RNA elements as novel drug targets for antiviral therapy

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SARS-CoV-2 comprises a linear positive-sense single-stranded RNA-genome of ~30 kb with known functional RNA structures located in the 5'-UTR (untranslated region), 3'-UTR and between the open reading frames (ORFs) 1a and 1b. Secondary or higher order RNA structures in the genome of coronaviruses can operate as regulatory cis-acting elements through RNA-protein or RNA-RNA interactions and serve essential functions in viral protein synthesis or replication. In the light of the SARS-CoV-2 pandemic, the genome of coronaviruses and its regulatory RNA elements got into the focus as novel drug targets for antiviral strategies. These RNA elements are conserved among the SARS-CoV-2 variants (of concern) and also between different coronaviruses. RNA-targeting small molecules offer an alternative strategy to directly target and alter RNA structures, by binding specifically to the viral genome and inhibiting its regulatory function e.g., during translation. Using SARS-CoV-2 as a blueprint, we focus on one essential RNA structure within the frameshift-stimulating region, namely the pseudoknot. The pseudoknot is located in the overlapping region between ORF1a and ORF1b and coordinates the essential -1 ribosomal frameshift during viral genome translation. Combining bioinformatics, in vitro and in cell methods as well as new advances in formulation of nano-particles, we performed a basic characterization of potential candidates targeting the SARS-CoV-2 pseudoknot. We found that different lead compounds show RNA binding in vitro and reduce -1 programmed ribosomal frameshift in cell, giving important insights into RNA-targeting drug development.

POSTER NUMBER: 48

In vitro reconstitution of active pre-mRNA 3'-end cleavage and polyadenylation machinery

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The nascent 3'-end mRNA maturation requires endonucleolytic cleavage and polyadenylation, processes called cleavage and polyadenylation (CPA). Reactions are 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. Majority of the human genes harbor at least two polyadenylation sites that are selected during alternative polyadenylation (APA) events. Depending on the localization APA can result in formation of different mRNA isoforms with various coding potentials, altered length of 3'UTRs and stability. Thus, APA contributes to gene expression variability.

We are interested in the mechanism of 3' end selection. Currently, we work on in vitro reconstitution of the 3'-end processing machinery via Sf9 insect cells/baculovirus expression system. Obtained proteins together with viral RNA substrates will be used in in vitro pre-mRNA cleavage assay. Moreover, we perform cleavage in HeLa nuclear extract testing various conditions.

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.

POSTER NUMBER: 49

Sf insect cell lines culturing for purification of mammalian factors required for the pre-mRNA 3'-end maturation

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Production of recombinant proteins is an essential tool for understanding complicate biological processes. Baculovirus Expression Vector System (BEVS) allows to produce high yields of post-translationally modified proteins. It embraces natural phenomenon of baculovirus infecting fall armyworm (*Spodoptera frugiperda*) to propagate. In BEVS a baculovirus is constructed in a way that genes of interest are cloned under baculoviral promoters of highly expressed late genes encoding polyhedrin (polh) and p10 protein (p10). Then the engineered baculovirus is transfected into insect cells where recombinant proteins are produced.

We employed BEVS for production of mammalian proteins and protein complexes fundamental for the pre-mRNA 3'-end maturation. We are using two insect cell lines: Sf21 cell line derived from the pupal ovarian cells of *Spodoptera frugiperda*, and its clonal isolate Sf9. The culture initiates from adherent, than cells are transferred to suspension for virus propagation or protein synthesis. According to our observations Sf21 cells are more efficient in transfection with baculovirus compared to Sf9 cell line.

POSTER NUMBER: 50

Activation kinetics of promoters in their interaction with enhancer elements.

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Each cell within a multicellular organism shares the same genome, but due to differential utilization of the genomic information, numerous different cell types are generated. Transcription levels are tightly regulated through the interplay of promoters and enhancers. However, even though transcription of DNA into RNA plays a key role in gene expression, it is a highly discontinuous process in nature. RNA production occurs in short bursts of transcription, separated by periods of apparent inactivity. Enhancers and promoters, being key drivers of transcription, are involved in modulating the transcriptional burst, however their role is not fully understood.

For my Ph.D. project, I am interested in studying the differences in bursting kinetics of promoters when they interact with diverse enhancer elements at different distances. Therefore, I am using a novel synthetic locus in mouse embryonic stem cells, to study the effect of the same enhancer at different distances from the same promoter. In addition, I will compare the bursting kinetics of different enhancers with different intrinsic strength.

In my work, I use live cell imaging to visualize and track transcriptional bursts over long periods of time to characterize their parameters, including the frequency of occurrence and the duration of the burst. To complement the analysis, I am applying single molecule RNA Fluorescence in situ hybridisation (FISH) to determine the amount of RNA molecules that are being transcribed at the transcription start site (burst size). The goal of my study is to shed some light on the correlation between bursting behaviour and intrinsic strength and location of the enhancer element. My results will improve our understanding of transcriptional regulation with a specific view on bursting kinetics.

POSTER NUMBER: 51

More than a splicing factor: exploring the cellular functions of ZNF830

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Intron-binding complex, composed of five proteins: AQR, XAB2, PPIE, ISY1, and ZNF830, is simultaneously recruited to the spliceosome during the Bact-complex formation. It is essential for cell function, mediating the transition of the spliceosome to a catalytically active state, and interestingly, also contributes to DNA damage repair. While the contribution of some complex components (AQR and XAB2) to these processes

have been well-characterised, the functions of other components remain less understood.

Here, we investigated the cellular functions of ZNF830, which, in addition to being part of the intron-binding complex, can also bind DNA. This indicates the potential of ZNF830 to influence gene expression at both the transcriptional and post-transcriptional levels. To examine ZNF830's effects on gene expression, we analysed the changes in RNA expression and splicing following ZNF830 knockdown. While the ZNF830 knockdown had minimal impact on global splicing patterns, there was a group of transcripts undergoing alternative splicing which was specifically enriched for DNA repair pathways. By modulating the splicing of DNA repair-related genes, this could indicate an enhancing role of ZNF830 in DNA repair function.

Looking at the binding position of ZNF830 by iCLIP, we detected substantial ZNF830 binding to the 5' and 3' untranslated regions (UTRs) of specific transcripts, alongside the expected branchpoint motifs. Notably, ZNF830 binding to 3' UTRs correlates with mRNA destabilisation of target transcripts, indicating a role for ZNF830 in mRNA stability.

Surprisingly, by analysing ChIP datasets, we found that ZNF830 binds DNA predominantly at transcription start sites of genes involved in chromatin organisation, cell division as well as DNA damage response, splicing, and mRNA decay.

Together, our findings suggest a dual role of ZNF830 in regulating splicing and RNA processing, by mediating the expression of spliceosomal and RNA processing machinery at the level of transcription, while also directly engaging in splicing and RNA processing at the transcript level.

POSTER NUMBER: 52

Studying possible differences in functions of LINC00116 or LINC00116-encoded Mitoregulin peptide in B-cell lymphoma cells

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Long non-coding RNAs (lncRNAs) are defined as RNAs longer than 200 nucleotides that are not translated into proteins. However, a number of lncRNAs have actually been shown to encode small peptides with significant functions in cells. One such lncRNA is LINC00116, which encodes a 56-amino-acid mitochondrial peptide Mitoregulin (MTLN). MTLN was shown to be involved in lipid homeostasis, energy metabolism and oxidative stress. The LINC00116 transcript has been reported to show higher levels in B-cell lymphomas compared to B lymphocytes. However, the role of both LINC00116 and MTLN in B-cell lymphoma is unknown.

The aim of this project is to investigate whether LINC00116 and MTLN have distinct cellular functions in B-cell lymphoma cells. We have shown that MTLN protein is overexpressed in Hodgkin lymphoma (HL) cells compared to normal B lymphocytes. We also demonstrated that LINC00116 is a direct target gene of miR-

17 family using luciferase assay. Subsequently, we prepared two overexpression constructs to selectively overexpress LINC00116 or MTLN peptide in L428 HL cells using lentiviral vectors. We confirmed LINC00116 overexpression by qRT-PCR, and MTLN overexpression by Western Blot. The lentiviral vectors contained the gene encoding GFP, which allowed cytometric analysis of transduced cells within 21 days. The percentage of L428 cells with overexpressed MTLN decreased compared to cocultured wild-type cells. This decrease was not observed when we overexpressed LINC00116 without MTLN-coding potential or in control EV-transduced L428 cells

In conclusion, we observed decreased growth of HL L428 cells with selectively overexpressed MTLN, and not with overexpressed LINC00116. Further studies to demonstrate possible differences in LINC00116 and MTLN functions in B-cell lymphoma are ongoing.

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POSTER NUMBER: 53

Centriolar satellites are sites of translation of centrosomal and ciliary proteins

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Centriolar satellites are small cytoplasmic particles found in the vicinity of centrioles and basal bodies in vertebrate cells. Numerous studies have linked satellites to a variety of cellular processes associated with centrosome and cilium biogenesis; however, the underlying molecular mechanisms are still poorly understood.

Here, we present an alternative model for satellites not as transport modules involved in centrosomal and ciliary trafficking but as sites of translation of centrosomal and ciliary proteins. Starting point for our analysis was our identification of a *Drosophila* ortholog of PCM1, named Combover or CMB. Like PCM1, Combover localizes to cytoplasmic foci containing centrosomal proteins and its depletion or mutation resulted in signature centrosomal and ciliary phenotypes. Satellites, then, are conserved beyond vertebrates. Strikingly, however, the concentration of satellites near centrosomes and cilia was not a conserved feature and therefore dispensable for satellite function. In order to understand CMB function, we performed proximity interaction analysis in both S2 cultured cells and whole flies, revealing not only centrosomal and ciliary proteins, but also RNA binding proteins and proteins involved in quality control. Follow-up work, both in *Drosophila* and in vertebrate cultured cells, found satellites to be associated with centrosomal and ciliary mRNAs, as well as evidence for protein synthesis occurring directly at satellites. Their concentration near centrosomes in vertebrates, especially in prophase/prometaphase, appears to be a consequence of co-translational trafficking of satellite client proteins such as PCNT, a process in which PCM1 may not be directly involved.

Given that PCM1 depletion is known not to affect overall protein levels we propose that satellites instead facilitate the coordinate synthesis of centrosomal and ciliary protein complexes. Such coordinate synthesis may explain the pleiotropic but overall rather weak phenotypes associated with PCM1 dysfunction compared to the centrosomal and ciliary clients whose assembly it promotes.

POSTER NUMBER: 54

Uncovering tsRNA Roles in Plant Stress: A Bioinformatic Exploration Across Species

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The study of small RNAs (sRNAs) is critical in plant biology, as due to their sessile nature, plants are continuously exposed to various biotic and abiotic stress factors, which severely affect their growth, productivity and quality. In response to these stresses, plants reprogram their transcriptional activities. Understanding how endogenous regulators and environmental factors interact during plant development and adaptation to stress is a longstanding challenge in plant science.

Advanced high-throughput sequencing technologies have greatly expanded the scope of small non-coding RNAs. One example is tRNA-derived small RNAs (tsRNAs), produced by enzymatic cleavage of tRNAs. Despite the complexity of the tsRNA population, these molecules can be primarily categorized into short fragments (~13-28 nt) and tRNA halves (~30-40 nt). Recent studies have shown that tsRNAs can regulate gene expression through various mechanisms, including transcription inhibition, RNA degradation, and translation regulation.

Although tsRNA expression changes under stress have been reported in plants, the mechanisms regulating their production remain unclear. This study aims to shed light on this aspect by adopting a comprehensive analysis of publicly available sRNA sequencing datasets from plant stress experiments. Covering over 60 species, spanning monocots and dicots, and a wide range of abiotic and biotic stresses, this dataset provides a unique opportunity to explore both conserved and stress-specific regulatory patterns of tsRNAs in plants. Before specifically focusing on stress-responsive tsRNAs, we first characterized the general landscape of tRNA-derived sequences across the projects. This included analyzing the conservation of parental tRNAs across species, overall tsRNA accumulation patterns, and conserved cleavage sites. This initial characterization provides a reference framework to better understand the specificity and regulation of stress-related tsRNAs.

The general overview of the expression of stress-responsive tsRNAs reveals a complex scenario, with differential expression depending predominantly on the type of stress, but also being sensitive to tissue, time and intensity of stress exposure. We categorized these sequences based on the generality or specificity of their response, allowing us to further investigate their features, including the tRNA isotypes and classes most consistently associated with stress responses. This classification also helps us gain insights into the structural characteristics of stress-responsive tsRNAs.

Taken together, this study leverages the wealth of available sequencing data to generate a global expression map of tsRNAs in plants under stress conditions, providing a valuable multispecies resource for future functional analyses.

POSTER NUMBER: 55

Deciphering regulation and functional conservation of dormant ribosomes

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Cellular quiescence is a reversible state of dormancy in which cells actively repress energy-intensive processes such as transcription and translation. As protein synthesis is one of the most energy-consuming processes in a cell, the ribosome plays a pivotal role in this state: to maintain quiescence, cells suppress ribosomal activity while maintaining a stable pool of ribosomes, enabling rapid exit from quiescence when needed.

We recently identified a novel class of dormant ribosomes stabilized by a conserved set of four factors in zebrafish and *Xenopus* eggs (Leesch et al., 2023). These factors block ribosomes in the egg and are released upon embryonic development to permit the upregulation of translation. While the composition and function of these dormant ribosomes have been characterized in fish and frogs, the mechanisms underlying their reactivation and their existence and roles in mammals remain poorly understood.

To address this, we expressed the human ortholog of the ribosome dormancy regulator DAPL1 in human cells, which led to dormant ribosome formation in a mammalian context. We found that DAPL1 stability depends on its association with the ribosome: mutations that disrupt this interaction lead to DAPL1 degradation via the proteasome. This suggests that factors involved in ribosome reactivation promote DAPL1 destabilization. To uncover such 'release' factors, we performed a genome-wide CRISPR screen using a FACS-based approach to enrich for cells with elevated DAPL1 levels. First results from our screen point towards components of the core translation machinery as regulators of DAPL1 stability and potential mediators of ribosome dormancy release.

Intriguingly, endogenous Dapl1 expression in mammals is restricted to quiescent cell types, including naïve T cells. Previous studies implicated DAPL1 in modulating CD8⁺ T cell activity, though its role in ribosome dormancy was unexplored. We show that DAPL1 binds ribosomes in naïve T cells and is released upon T cell activation, supporting a model in which DAPL1 mediates ribosome dormancy in the mammalian immune system. Using Dapl1 knockout mice, our data suggest impaired preservation of the core translation machinery in naïve T cells, reinforcing the functional significance of DAPL1 in cellular quiescence.

Together, our findings support a conserved role for dormant ribosomes in maintaining quiescence from fish to mammals, revealing a previously underappreciated layer of translational regulation in dormant cells.

POSTER NUMBER: 56

Global analysis of stress response mediated by miRNAs in plants

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miRNAs are a group of small RNAs (around 20-24 nt) that do not encode proteins and act as regulators of gene expression at the transcriptional and post-transcriptional level through interactions based on sequence complementarity. In plants, these molecules play a key role in the regulation of processes related to development and stress response. Although the function of these molecules in plant-environment interactions has been extensively studied, the type of miRNA-based regulatory architectures used by plants to cope with stress has not been as extensively explored [1, 2].

Here, we address this aspect globally, using public data from multiple and diverse plant species. This approach involved searching for and downloading miRNA libraries from studies related to stress response in plants, followed by their processing and filtering according to established criteria of sequencing depth and sequence length. From these libraries, count matrices were generated, which were subsequently used to carry out a differential expression analysis for each of the stress events analyzed. sRNA sequences with significantly differential expression were annotated for the identification of miRNAs and their grouped into families. As a result, miRNA families were classified into three categories based on their stress response range: General, Intermediate, and Specific. General miRNAs seem to predominantly regulate transcription factors involved in broader biological processes, while those with a narrower response range are implicated in more diverse and specific functions. This work offers an innovative and integrative view of the plant response (mediated by miRNAs) to unfavorable or changing environmental conditions.

References:

[1] Sanz-Carbonell, A., Marques, M. C., Bustamante, A., Fares, M. A., Rodrigo, G., & Gomez, G. (2019). Inferring the regulatory network of the miRNA-mediated response to biotic and abiotic stress in melon. *BMC plant biology*, 19(1), 78. <https://doi.org/10.1186/s12870-019-1679-0>

[2] Sanz-Carbonell, A., Marques, M. C., Martinez, G., & Gomez, G. (2020). Dynamic architecture and regulatory implications of the miRNA network underlying the response to stress in melon. *RNA biology*, 17(2), 292–308. <https://doi.org/10.1080/15476286.2019.1697487>

POSTER NUMBER: 57

Computational design of a programmable sequence-specific ssRNA binding protein

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Detection and regulation of ssRNA is of great interest in synthetic and cell biology. In this context, it is highly desirable to develop a programmable ssRNA binding protein. Taking inspiration from natural RNA binding proteins, we propose to develop a ssRNA binding protein that is highly programmable.

We extracted RNA-binding motifs from Pumilio and FBF homology (PUF) proteins. Each of them was repeat-

ed multiple times in silico to create a highly repetitive structure. The protein backbone structure was refined with RFDiffusion, and the amino acid sequences were designed by LigandMPNN with RNA homopolymers as the ligand.

The experimental screening of the designed proteins yielded one binder with low nanomolar affinity to poly(U) and two weak binders to poly(A) and poly(U).

Based on the comparison between the computational scoring metrics and the experimental results, our current effort is focused on the next round of the design campaign.

POSTER NUMBER: 58

Sequence-Specific DNA Hydrolysis and Partial Reverse Splicing by an ORF-Less Bacterial Group IIC Ribozyme In Vitro

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Group II introns are mobile ribozymes that invade genomes through reverse splicing and reverse transcription. Canonical group II introns encode an intron-encoded protein (IEP) within their open reading frame (ORF). The IEP and intron form a ribonucleoprotein (RNP) complex that facilitates intron splicing and enables mobility. Structurally, group II introns are classified into three major subclasses: IIA, IIB, and IIC, with bacterial group IIC introns considered the most ancestral due to their simpler organization.

In this study, we investigate hydrolytic endonucleolytic ribozyme 1 (HYER1), an ORF-less group IIC intron from *Anoxybacillus pushchinoensis*. Recent research has shown that HYER1 exhibits specific single-stranded DNA (ssDNA) hydrolysis activity. Our findings expand HYER1's catalytic repertoire by demonstrating its ability to perform partial reverse splicing into ssDNA in vitro. These results provide new insights into the catalytic potential of ORF-less group II introns and contribute to a broader understanding of their mobility.

POSTER NUMBER: 59

Role of multivalent nucleotide sequences in mRNA coregulation

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Protein homeostasis is essential for the proper functioning of cells and can be regulated by complementary mechanisms at the level of transcription, RNA metabolism, and proteins. Proteins containing intrinsically disordered regions (IDRs) are particularly dosage-sensitive and are therefore under tighter regulation (Babu et al, 2011). Such regions are common in RNA-binding proteins, and their disruptions are linked to various diseases (Järvelin, 2016), including neurodegeneration. A recent study (Faraway et al., 2023) described a novel mechanism that co-regulates IDR-containing proteins and their mRNAs, termed interstasis. Here, GA-rich multivalent mRNAs encoding IDR-containing proteins can become retained in nuclear speckles, thereby establishing a negative feedback loop that regulates the expression of specific IDR-containing proteins when their levels rise. Notably, the GA-rich multivalency is enhanced by codon biases in the coding sequences of these mRNAs. Other examples of important multivalent sequences include AU-rich, GU-rich and CA-rich multivalent elements in the 3'UTRs, which regulate mRNA stability (Hong & Jeong, 2023).

We aimed to characterise the RNA multivalency types across different transcript regions to be able to investigate the relationships between these regions within transcripts. We therefore analysed RNA multivalency across the coding sequences, introns, and 3' untranslated regions (3'UTRs) of human RNAs. We performed transcriptome-wide peak calling to identify and classify distinct multivalency types and analyzed their co-occurrence. We found that specific transcript groups exhibit a coupling of distinct multivalent regions in CDS and 3'UTRs. We also integrated the transcript's multivalency potential with structural, functional and cell-location annotations of the encoded proteins, which gained insights into the potential biological relevance of multivalent RNA regions.

Together, our analysis provides new insights into the post-transcriptional regulation through RNA multivalency and indicates that this might broadly contribute to the homeostasis of IDR-containing proteins.

Babu M. M., et al. Intrinsically disordered proteins: regulation and disease. *Current Opinion in Structural Biology* 21, 432–440 (2011) doi: 10.1016/j.sbi.2011.03.011

Järvelin A. I., et al. The new (dis)order in RNA regulation. *Cell Communication and Signaling* 14, (2016) doi: 10.1186/s12964-016-0132-3

Faraway R., et al. Mutual Homeostasis of Charged Proteins. *bioRxiv* (2023) doi: 10.1101/2023.08.21.554177

Hong D. & Jeong S. 3'UTR Diversity: Expanding Repertoire of RNA Alterations in Human mRNAs. *Molecules and Cells* 46, 48–56 (2023) doi: 10.14348/molcells.2023.0003

POSTER NUMBER: 60

Gas-sensing riboceptors

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In the scientific literature, direct gas-sensing biomolecules are primarily metalloproteins (gasoreceptors) such as the heme-based NO (nitric oxide)-sensing gasoreceptors soluble guanylate cyclase and DGCR8/DROSHA (in mammals), and the O₂-sensing gasoreceptors FixL kinase and Dos phosphodiesterase in microorganisms.^{1,2} However, if the RNA world hypothesis is true, then one of the first gas-sensing biomolecules is potentially RNA (either riboswitches or ribozymes), since gases probably existed before the origin of nucleic acids, and RNA probably did not form in a vacuum.^{2,3} The identity of gas-sensing riboceptors (allosterically-regulated ribozymes) capable of binding and sensing various essential and nonessential gasotransmitters or gaseous/volatile signaling molecules are still unknown. The experimental identification of gasoriboceptors can potentially help not only to understand the origin of environmental gas sensing and autocatalytic ribozyme mechanisms in the RNA world hypothesis, but also of the following postulates of gasocrine signaling. 1) All living organisms composed of one or more cells require gasocrine signaling to sense, communicate, survive and propagate. 2) Gasocrine signaling mediated via gasoreceptor proteins (or perhaps riboceptors) is the most essential cellular and inter-organism signaling. 3) All cells and acellular entities arising from or replicating in pre-existing cells require gasocrine signaling.

References:

Aono S. *Gas sensing in cells*. Royal Society of Chemistry. Great Britain. 2017.

Anbalagan S. *Gas-sensing riboceptors*. *RNA Biol.* 2024. DOI: 10.1080/15476286.2024.2379607

Atkins J.F, Gesteland R.F, Cech T. *The RNA World*. CSHL Press USA. 2006

POSTER NUMBER: 61

Exosomal small RNA profiling in first-trimester maternal blood explores early molecular pathways of preterm preeclampsia

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Preeclampsia (PE) is a severe pregnancy complication marked by new-onset hypertension and proteinuria, often linked to fetal intrauterine growth restriction (IUGR). Early diagnosis is crucial, but the heterogeneity of PE complicates early identification. This study explores maternal plasma-derived exosomal small RNAs in preterm PE with IUGR as a potential non-invasive diagnostic tool and a source of insights into the complex pathology of this PE subtype. Exosomes were isolated from first-trimester plasma of women who later developed PE with IUGR (n=6) and healthy controls (n=14). Small RNA content was analyzed via next-generation sequencing and validated with qRT-PCR. Over 2700 small RNAs were identified, primarily from RNA interference pathways. Sixteen microRNAs were upregulated in PE, while Piwi-associated RNAs showed mixed expression changes. Gene ontology analysis highlighted pathways crucial for placentation and embryonic development. Validation experiments identified hsa_piR_016658 as a promising biomarker for preterm PE with IUGR.

POSTER NUMBER: 62

Assessing the function of conserved cryptic splice sites in Hnrnph1 introns

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The regulatory role of intronic sequences located far from exons remains poorly explored. These sequences encompass various regulatory elements, including cryptic splice sites. Such cryptic sites can act as decoys that compete with the canonical splice sites for spliceosomal recruitment and thus interfere with splicing of nearby exons. Only a few specific cryptic splice sites have been interrogated in depth, and therefore the regulatory mechanisms behind most such sites and their functions are still to be thoroughly examined.

We analysed data produced by iCLIP of spliceosomal component proteins SmB and PRPF8, which identify the assembly sites of early or late spliceosomal complexes across the transcriptome. This identified thousands of major intronic binding peaks far from exons, which tend to overlap with cryptic splice site elements. We focused on cryptic 5' sites in the introns of Hnrnph1 pre-mRNA, which were identified both by iCLIP of SmB and PRPF8. Further analysis has shown that the introns containing such sites tend to be slowly spliced according to RNA-seq data. Imaging combined with RT-qPCR following nuclear fractionation confirmed that the intron-retained transcripts are retained in the nucleus. Global iCLIP data further demonstrated extensive RNA-binding protein (RBP) occupancy on the retained intron.

Evolutionary analysis revealed that the cryptic 5' site sequence in intron 5 is highly conserved across placental mammals, with intron retention documented by RNA-seq across mammalian species. Additionally, a substantial portion of the remaining sequence of the intron 5 exhibits very high evolutionary conservation.

To functionally assess the role of the cryptic 5' site in intron 5, we deleted the site from both alleles of two mouse embryonic stem cell (mESC) lines. This led to increased intron splicing efficiency, a corresponding rise in the correctly spliced isoform, and elevated hnRNPH1 protein expression. Moreover, we identified widespread splicing changes across the transcriptome. Analysis of these cell lines, along with minigene experiments are used to investigate how the cryptic 5' site and associated retention mechanism serve to regulate hnRNPH1 expression, and whether the intron-retained transcripts may also play additional functions in gene expression regulation.

POSTER NUMBER: 63

Functional role and diagnostic application of differentially expressed miRNAs in Lung Adenocarcinoma Brain Metastasis and Glioblastoma.

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Background: Brain metastases (BM) represent the largest proportion of malignant lesions affecting the central nervous system (CNS) and originate from lung cancer in 40–50% of cases. Lung adenocarcinoma (LUAD) – the most common pathological subtype of non-small cell lung cancer (NSCLC) – leads to BM development in approximately 25% of cases. The incidence of BM is increasing and often presents synchronously with the primary tumor. Furthermore, distinguishing between Glioblastoma (GBM) – the most aggressive primary malignant brain tumor – and solitary BM remains a significant challenge due to the limitations of conventional MRI-based imaging, as these tumor types may exhibit overlapping morphological characteristics. Consequently, the identification of novel biomarkers has become crucial for achieving more accurate diagnoses and improving patient survival outcomes. Given the pivotal role of microRNAs (miRNAs) in regulating gene expression, their clinical potential as biomarkers for tumor diagnosis and prognosis is highly promising. Identifying specific miRNAs could provide a novel approach for distinguishing between BM, GBM, and control samples.

Aim: We aimed to identify microRNAs that exhibit distinct expression patterns in intraoperative malignant brain tissue samples of LUAD-BM and GBM, as well as in tumor-free control brain tissue samples. A key objective was to identify miRNAs whose expression changes are associated with metastatic processes and may contribute to the reliable identification of brain metastases while distinguishing them from other types of brain tumors.

Methods: We used next-generation RNA sequencing (RNA-seq) to map miRNA expression profiles of 6 LUAD-BM, 6 GBM, and 6 non-cancerous human brain tissue samples. Normalized data were evaluated with the iDEP.96 tool. The validation of differentially expressed (DE) miRNAs was performed using RT-qPCR. Statistical analysis included the identification of significantly altered miRNAs using the Mann-Whitney U test, and assessed diagnostic efficiency via ROC analysis. The miRNA-centered target and functional enrichment analyses were performed using the miRNet tool.

Results: RNA-seq analysis revealed 229 DE miRNAs between LUAD-BM and control samples and 46 between LUAD-BM and GBM. The Mann-Whitney U test confirmed that upregulated miR-200c-5p, miR-141-5p, miR-200a-5p, miR-375-3p, miR-196b-5p, miR-130b-3p, miR-200c-3p, miR-210-3p, miR-503-5p and downregulated miR-383-5p, miR-129-2-3p, miR-410-3p, miR-9-5p, miR-195-5p, miR-138-2-3p exhibited significant differences between LUAD-BM and control samples. Furthermore, our analysis demonstrated that miR-200c-5p, miR-141-5p, miR-200a-5p, miR-375-3p, miR-383-5p, miR-129-2-3p, miR-410-3p, and miR-9-5p can reliably distinguish between LUAD-BM and GBM samples. ROC curve analysis further evaluated the diagnostic potential of these miRNAs. Our functional annotation analysis revealed that the DE miRNAs identified by NGS are key regulators of tumorigenesis and metastasis, which confirms the pathophysiological role of miRNAs in various tumor processes.

Conclusions: We successfully validated 15 miRNAs that can significantly distinguish LUAD-BM from control

samples. Furthermore, we identified an eight-miRNA panel that exhibits outstanding sensitivity and specificity in differentiating LUAD-BM, GBM, and control brain tissue samples. In combination, these miRNAs have the potential to be used to diagnose LUAD-BM and to distinguish it from GBM with 100% sensitivity and specificity.

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POSTER NUMBER: 64

Structural characterization of tRNA binding by the human FTSJ1-THADA methyltransferase complex

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tRNAs are extensively post-transcriptionally modified at various positions, with the anticodon stem loop (ASL) being a modification hotspot. Modifications in the ASL are predominantly involved in the translational decoding process, and their absence is often associated with various human diseases. Human FTSJ1 is a tRNA 2'-O-methyltransferase (MTase) that modifies the ribose 2'-OH moiety of residues 32 (Nm32) and 34 (Nm34) in the ASL of 21 reported tRNAs. FTSJ1 forms a complex with either THADA to methylate residue 32 or with WDR6 to methylate residue 34. Of the 21 described FTSJ1 targets, 17 tRNAs carry only the THADA dependent Nm32 modification, two tRNAs have only the WDR6 dependent Nm34 methylation, whereas tRNA^{Phe} and tRNA^{Trp} are modified at both positions. Mutations in human FTSJ1 are associated with X-linked intellectual disability and cancer. The exact molecular mechanisms that lead to tRNA selection and site specificity by the THADA-FTSJ1 complex remain unknown. To address this issue, we purified the heterologous expressed human FTSJ1-THADA complex to homogeneity. We use single particle cryo-EM to determine the high-resolution structure of FTSJ1-THADA in different states and with different tRNA species. Furthermore, we used complementary biochemical and biophysical approaches to characterize the binding specificity and methylation activity towards different tRNA substrates in vitro. Further, we mapped and analysed residues on THADA and FTSJ1 that are involved in tRNA binding and protein-protein interactions. Based on our structure, we are able to demonstrate how THADA and FTSJ1, orchestrate the deformation of the ASL and position the ribose into the active site.

POSTER NUMBER: 65

Identification of deregulated miRNAs and mRNAs involved in glioblastoma development using next-generation RNA sequencing

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Background: Glioblastoma (GBM) is considered the most common and lethal cancer of the adult brain, remaining incurable with a median survival time of only 15 months. Treatment options are limited, clinicians lack efficient diagnostic, prognostic and predictive markers. In recent years, molecular biology research has increasingly focused besides of mRNAs on the study of miRNAs. These RNA molecules regulate essential cellular processes at the post-transcriptional level, therefore, alterations in their normal balance and expression levels can lead to the development of various diseases, including tumor formation.

Aim: In our study we aimed to perform combined expression profiling of miRNAs and mRNAs of control and malignant brain tissue samples of GBM to identify those up- and downregulated miRNAs and mRNAs that can be associated with tumor development and can be used as potential diagnostic biomarker.

Methods: Total RNA isolated from brain tissue samples of 5 control individuals and 5 GBM patients (GPs) were used for high-throughput RNA sequencing (RNA-Seq). The validation of significantly deregulated miRNAs and mRNAs was performed using RT-qPCR. Statistical analysis included the Mann-Whitney U test, and ROC analysis. The functional enrichment analyses were performed using the Network Analyst and miRNet tool.

Results: Bioinformatic (iDEP 96) analysis demonstrated significant upregulation of 35 miRNAs and 365 mRNAs, and significant downregulation of 82 miRNAs and 1225 mRNAs in tissue samples of GPs compared to control samples on the base of the results of RNA-Seq. The 5 miRNAs (hsa-miR-196a-5p, hsa-miR-21-3p, hsa-miR-10b-3p, hsa-miR-383-5p, hsa-miR-490-3p) and 14 mRNAs (E2F2, HOXD13, VEGFA, CDC45, AURKB, HOXC10, MYBL2, FABP6, PRLHR, NEUROD6, CBLN1, HRH3, HCN1, RELN) selected for validation by RT-qPCR were proved to be deregulated in GPs. The functional annotation analysis revealed that those miRNAs and mRNAs that showed altered expression in GPs are play important role in tumorigenesis.

Conclusions: A miRNA panel and numerous deregulated mRNAs identified in our study may play a crucial role in tumor development, bringing us closer to a more precise understanding of the disease and assisting in distinguishing GBM patients from healthy controls.

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POSTER NUMBER: 66

Collective dosage regulation of condensation-prone proteins influences cellular physiology and proliferation

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Dosage regulation of proteins containing intrinsically disordered regions (IDRs) is key for maintaining proteostasis. These proteins have a tendency to localise to membraneless condensates. Specifically, proteins containing arginine-rich mixed charge domains (R-MCDs) tend to localise into nuclear speckles and their accumulation was shown to increase speckle cohesion. Recently, our group has shown that upon initial stages of R-MCD accumulation, speckles also selectively retain highly multivalent mRNAs, especially those that are rich in purines (GA-rich mRNAs), which often encode R-MCD containing proteins themselves. This mechanism of selective speckle sequestration of GA-rich mRNAs, termed interstasis, prevents their nuclear export and is predicted to regulate the collective dosage of R-MCD-containing proteins.

Here, we aimed to further evaluate the effects of long term R-MCD accumulation and interstatic regulation on the homeostatic feedback. We created multiple inducible cell lines expressing R-MCD in fusion with fluorescent protein mGreenLantern, where R-MCD is either encoded by high or low-multivalent mRNA. Using RNA sequencing, confocal microscopy and cell proliferation assays, we elucidate the time-dependent effects of R-MCD expression on cell physiology and proliferation. Comparing cell lines with high and low multivalency mRNA, we demonstrate the regulatory role that the multivalency in mRNAs plays to limit the toxicity of R-MCD accumulation. Together, we examine the significance and mechanisms of interstasis in maintaining healthy cell physiology.

POSTER NUMBER: 67

DyRNA Thermometry allows to monitor position specific structural changes in folded RNAs

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RNA molecules not only contain linear sequence information, but they also fold into secondary structures and can attain complex three-dimensional shapes. Folded RNA molecules are dynamic and they can switch between different structural conformations. The low number of robust, simple and precise methodology to track these structural changes in RNA molecules limits our efforts to understand these dynamic processes. Here, we present DyRNA Thermometry, a fluorescence-based technique for monitoring RNA conformational stability in vitro. Leveraging the principle of the so called "temperature-related intensity changes" (TriC) effect, this fluorescence based method allows to measure the thermostability of individual position in RNA molecules. In this study, we tested the applicability of different fluorescent probes and benchmarked DyRNA Thermometry by monitoring the specific process of unfolding in small RNA hairpins. Furthermore, we show

that the method is able to track domain-specific conformational changes in RNAs and sense ligand-induced conformation changes in different families of riboswitches. As the method is highly sensitive and requires little sample quantities it is highly suitable for high-throughput screening, needed in various biotechnological applications and the discovery pipelines for RNA-based therapeutics. The approach offers a versatile tool for structural studies of RNA, providing new insights into RNA stability and identifying optimal experimental conditions. Hence, DyRNA Thermometry's ability to detect subtle conformational shifts in RNA molecules provides a novel and complementary tool for RNA researchers in academia and industry.

POSTER NUMBER: 68

Adenosine (de)methylation on the crossroads of RNA and DNA synthesis

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Eukaryotic mRNAs are subject to significant chemical modifications that exhibit a multi-layered regulation of transcript metabolism. Among these, N⁶-methyladenosine (m⁶A) and N⁶-2'-O-dimethyladenosine (m⁶Am) are two prevalent modifications in higher eukaryotes [1]. The dynamics of these modifications are tightly governed by dedicated machineries, the perturbation of which destabilise cellular homeostasis. In a recent study using proximity-based labelling approach, we identified that m⁶A/m eraser protein – FTO – was proximally interacting with proteins in DNA replication and repair pathways [2]. To elucidate this further, in this study we performed subsequent protein interaction assays and genome wide CRISPR screening, where we identified multiple candidates involved at different levels of DNA synthesis. Additionally, we performed functional assays to further investigate the relevance of FTO in these processes. Preliminary results from these functional assays positively correlate with our observations from the high throughput screening approaches, highlighting the importance of the m⁶A/m modifying enzyme, FTO, in the maintenance of genome integrity.

POSTER NUMBER: 69

Importance of an N-terminal structural switch in the distinction between small RNA-bound and free ARGONAUTE

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ARGONAUTE (AGO) proteins bind to small non-coding RNAs to form RNA-induced silencing complexes. In the RNA-bound state, AGO is stable while RNA-free AGO turns over rapidly. Molecular features unique to RNA-free AGO that allow its specific recognition and degradation remain unknown. Here, we identify a con-

lined, linear region in Arabidopsis AGO1 and human Ago2, the N-coil, as a structural switch with preferential accessibility in the RNA-free state. RNA-free Arabidopsis AGO1 interacts with the autophagy cargo receptor AT11 by direct contact with specific N-coil amino acid residues whose mutation reduces the degradation rate of RNA-free AGO1 in vivo. The N-coil of human Ago2 co-purify with E3 ubiquitin ligases and members of the proteasome and has similar degron activity as the Arabidopsis AGO1 N-Coil dependent on residues in positions equivalent to those required for the Arabidopsis AGO1–AT11 interaction. These results elucidate the molecular basis for specific recognition and degradation of the RNA-free state of eukaryotic AGO proteins.

POSTER NUMBER: 70

Global analysis by LC-MS/MS of N6-methyladenosine and inosine in mRNA reveal complex incidence

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The precise and unambiguous detection and quantification of internal RNA modifications represents a critical step for understanding their physiological function. Only handful of marks can be detected by reverse transcription and sequencing, for some thanks to additional chemical conversions of isolated RNAs. One of the biggest challenges in the field still remains detection and quantification of m6A, m6Am and m1A modifications. Second intriguing and timely question remained to be addressed is the extent to which individual marks are coregulated or potentially can affect each other. Here we present an methodology and study of the detection and quantification of several key mRNA marks in human total RNA and mRNA. We show that the adenosine demethylase FTO primarily targets m6Am marks in noncoding RNAs in HEK293T cells. Surprisingly, we observe little effect of FTO or ALKBH5 depletion on the m6A mRNA levels. Interestingly, upregulation of FTO but not ALKBH5 is accompanied by an increase in inosine level in overall mRNA.

POSTER NUMBER: 71

Tackling the molecular function of human RNA demethylase ALKBH5

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Messenger RNA (mRNA) maturation is a highly intricate process involving multiple layers of regulation, which are closely tied to various quality control mechanisms. Among these regulatory elements, mRNA modifications have emerged as key modulators of gene expression, with N6-methyladenosine (m6A) being the most abundant modification in eukaryotic mRNA. The enzyme ALKBH5, an m6A demethylase, is conserved across species and plays a role in regulating m6A levels [1]. At the cellular level, the removal of m6A by ALKBH5 has been linked to mRNA processing, export, localization, stability, and translation. Despite multiple studies on ALKBH5, we are still lacking good understanding of its molecular function. Our recent interactome analyses revealed that ALKBH5 interacts with the core exon junction complex (EJC) components, peripheral proteins, several mRNA export factors and 3’ end mRNA processing factors. This is in agreement with previous study

showing that ALKBH5 depletion leads to poly(A) RNA accumulation in the cytoplasm [2].

To further tackle the mechanism of ALKBH5 in these events, we performed high-throughput CRISPR/Cas9 genetic interaction screening, where we observed pronounced synthetic lethality for EJC components and several splicing factors. Furthermore, ALKBH5 depletion led to dramatic changes in mRNA expression and processing. Also, ALKBH5 KO cells displayed alternative splicing (AS) and 3’ UTR phenotypes. Compared to our previous studies on the second m6A demethylase FTO, ALKBH5 appears to promote AS, causing the inclusion of exons, by possibly catalysing m6A demethylation at the 5’ termini of mRNAs. Overall, our results reveal the importance of ALKBH5 in pre-mRNA processing and export.

REFERENCES

1. Rajecka, V., Skalicky, T., & Vanacova, S. (2019). The role of RNA adenosine demethylases in the control of gene expression. In *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* (Vol. 1862, Issue 3, pp. 343–355). Elsevier B.V. <https://doi.org/10.1016/j.bbagr.2018.12.001>
2. Zheng, G., Dahl, J. A., Niu, Y., Fedorcsak, P., Huang, C. M., Li, C. J., Vågbo, C. B., Shi, Y., Wang, W. L., Song, S. H., Lu, Z., Bosmans, R. P. G., Dai, Q., Hao, Y. J., Yang, X., Zhao, W. M., Tong, W. M., Wang, X. J., Bogdan, F., ... He, C. (2013). ALKBH5 Is a Mammalian RNA Demethylase that Impacts RNA Metabolism and Mouse Fertility. *Molecular Cell*, 49(1), 18–29. <https://doi.org/10.1016/j.molcel.2012.10.015>
3. Yang, X., Triboulet, R., Liu, Q., Sendinc, E., & Gregory, R. I. (2022). Exon junction complex shapes the m6A epitranscriptome. *Nature Communications*, 13(1). <https://doi.org/10.1038/s41467-022-35643-1>

POSTER NUMBER: 72

RNAi induced gene silencing via poly(UG) tailed RNA in the satellite model organism *Pristionchus pacificus*

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In the nematode *Caenorhabditis elegans*, the terminal transferase RDE-3 adds a poly(UG) tail to the free 3’ end of RNA molecules generated by the action of primary siRNAs or piRNAs. The tailed RNA serves as a template for RNA dependent RNA polymerases (RdRp) to generate secondary siRNAs. In this iterative process, progressively shorter tailed RNAs are formed. In *C. elegans*, injection of poly(UG) tailed single stranded RNA(ssRNA) leads to RNAi-mediated gene silencing, thereby bypassing the need for the processing of double stranded RNA into siRNAs. We wondered if poly(UG) tailed ssRNAs could also be used for experimental gene knock down in nematodes where long dsRNA-mediated RNAi does not work reliably, such as the satellite model organism *Pristionchus pacificus*.

The injection of tailed RNAs targeting two endogenous genes (*eud-1*, *dpy-1*) and a transgene (*daf-1::TurboRFP*) into the gonads of *P. pacificus* worms led to gene-specific knockdown in the next generation and to newly generated poly(UG)-tailed RNAs that were shorter than the injected ones. Using poly(UG) tailed RNAs that were tagged by two point mutations, we showed that both endogenous and injected RNAs served as substrates for the formation of new tailed RNAs. Poly(UG)-tailed RNAs targeting a multi copy transgene were also detected independently of RNA injection, suggesting that the mechanism is endogenously employed in *P. pacificus*. We knocked out the one-to-one ortholog of *rde-3* in *P. pacificus* by CRISPR/Cas9 gene editing. All seven *rde-3* mutant alleles were maternal effect lethal. In *rde-3* mutant animals that were the progeny of

heterozygous mothers, poly(UG)-tailed RNAs were still detectable, presumably due to the maternal contribution of RDE-3 activity, which allowed these mutants to reach adulthood.

Unlike in *C. elegans*, in *P. pacificus* gene silencing was not strictly dependent on the presence of a poly(UG) tail. RNAs with a poly(CA) tail or without tail also induced gene-specific knockdown, although less efficiently, compared to the poly(UG) tailed RNA. To rule out the possible contribution of small amounts of dsRNA arising during the in vitro synthesis of the tailed RNAs, we repeated the experiments in an *rde-1* mutant background. In *C. elegans*, *rde-1* mutants are defective in early steps of the primary RNAi pathway and show increased expression of transgenes. *P. pacificus* has three *rde-1*-like genes. We knocked out all of them by CRISPR/Cas9, individually and in combination. Even the triple mutant still showed the gene knockdown effect for tail-less RNA. Consistent with *C. elegans*, we observed an increase in the expression of the *daf-1::TurboRFP* transgene.

Our results suggest that the poly(UG) tailed RNA-dependent RNAi enhancement mechanism described in *C. elegans* is conserved in *P. pacificus*, a nematode that is estimated to be phylogenetically separated from *C. elegans* by about two hundred million years but still belongs to the same major clade of nematodes (clade V). We are currently testing the existence of poly(UG) and other tailed RNAs in the human parasitic nematode *Strongyloides stercoralis*, and the rat parasite *Strongyloides ratti* that belongs to clade IV.

POSTER NUMBER: 73

Investigating RNA-RNA interactions in rotavirus genomic packaging

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Rotaviruses are pathogenic viruses that possess segmented genomes made up of eleven double-stranded RNA segments, each selectively packaged into individual virions. The exact mechanism of this assortment is unclear, however, it is becoming increasingly apparent that RNA-RNA interactions are involved in driving this assortment.

To determine which interactions may be involved in the assortment of the rotavirus genome, we employed RNA structure probing techniques. We used the sequencing of psoralen crosslinked, ligated, and selected hybrids (SPLASH) method to directly observe RNA-RNA interactions among rotavirus transcripts. This approach involves treating RNA with psoralen, which, upon UV irradiation, induces crosslinking, and subsequently sequencing the resulting hybrid RNA to map both intra- and intermolecular interactions. To analyse the SPLASH data, a bioinformatics pipeline was developed using the Nextflow workflow management software. The pipeline automates several steps, including data pre-processing, read alignment, and identification of putative RNA-RNA duplexes. Downstream analysis was conducted using the *rnaCrosslinkOO R* package that uses SPLASH data to constrain in silico RNA folding.

This ongoing work is focused on interrogating key differences in the formation of short-range and long-range RNA-RNA interactions, as well as the RNA contacts that arise during rotavirus transcription. Elucidating these interactions will shed light on the role of co-transcriptional RNA folding in the mechanism of genomic RNA assortment and facilitate the identification of key structural RNA motifs in rotaviruses.

POSTER NUMBER: 74

piRNA pathway beyond *Drosophila*: Argonaute expansion in *Bradysia*

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Argonaute proteins play crucial roles in small RNA-based gene regulation, yet their evolution and function outside model species remains largely unexplored. Here, we investigate their diversification in Sciaridae and Cecidomyiidae, two fly families with unusual chromosome inheritance mediated by programmed DNA elimination. By building molecular phylogeny of fly families within Bibionomorpha and Brachycera, we identify a substantial stepwise expansion of Piwi-like genes in the *Bradysia* genus (Sciaridae), potentially driven by directional selection. In *Bradysia coprophila*, these expanded Argonaute genes show female germline-biased RNA expression, and different PIWI clade Argonaute proteins show distinct perinuclear or nuclear localization patterns indicating the presence of diverse post-transcriptional as well as co-transcriptional piRNA pathway branches. By characterizing these newly identified Argonautes in *Bradysia coprophila*, we aim to uncover the functional roles and potential innovations in the piRNA pathway in a non-*Drosophila* species with unique germline chromosome biology.

POSTER NUMBER: 75

The role of interstasis in regulation of multivalent mRNAs and low-complexity domain containing proteins

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Precise protein dosage regulation is key to maintaining cellular homeostasis and thus for healthy cellular physiology. Recently, we reported a feedback mechanism that maintains a mutual homeostasis of condensation-prone nuclear proteins, termed interstasis (Faraway et al. 2023). The core feedback loop was induced by overexpression of R-MCD region of PPIG which led to accumulation of arginine-rich mixed-charge domains (R-MCDs). This led to sequestration of PPIG and GA-multivalent mRNAs, which encode R-MCDs in nuclear speckles. R-MCD-rich proteins accumulate in nuclear speckles with TRA2 proteins acting as dosage-sensitive effectors that promote GA-multivalent mRNA sequestration during interstasis.

Here, we ask whether interstasis can be induced through overexpression of other R-MCD peptides and other protein groups encoded by multivalent mRNAs. Firstly, we examine whether overexpression of R-MCD from SRSF11, encoded by GA-multivalent mRNAs also induced selective GA-rich mRNAs and protein sequestration in the nuclear speckles. Using light microscopy, we observe translocation of GA-rich mRNAs to the nucleus suggesting that SRSF11 can indeed induce interstatic dosage-regulation. Then, we examined the role of C- or GC-multivalent regions in interstasis. In particular, C-rich multivalent mRNAs tend to encode IDRs in

actin-binding proteins. Combining bioinformatics with experimental data, we ask whether multivalency can lead to coregulation of actin-binding proteins. Together, we investigate the role of interstasis in homeostatic control of related groups of IDR-containing proteins.

POSTER NUMBER: 76

Preserving the Message: isomiR Landscapes in Cells, EVs, and Protein Complexes

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Background:

MicroRNAs (miRNAs) exist not only in their canonical form but also as isoforms known as isomiRs, which differ in sequence length and nucleotide modifications. Distinct isomiR profiles have been observed across different cell types. Extracellular vesicles (EVs) play an important role in the exchange of RNA between cells and miRNAs can be selectively loaded into these vesicles. Additionally, the packaging of miRNA cargo as well as the formation of miRNA – protein complexes can have a protective function and prevent secreted RNAs from degradation. While canonical miRNA loading has been studied extensively, it remains unclear whether isomiR profiles differ between intracellular and extracellular environments.

Aim:

This study aims to systematically characterize isomiR profiles across three distinct RNA compartments in an in vitro cell culture model: (i) intracellular RNA, (ii) extracellular vesicle-associated RNA, and (iii) protein-bound extracellular RNA.

Methods:

RNA was isolated from four different cell lines including intracellular RNA, and RNA derived from corresponding EV and protein fractions. Small RNA sequencing was used to analyze isomiR and miRNA composition in each fraction. Expression Profiles were investigated on three different levels using a specifically developed pipeline for isomiR analysis (isomiRScope):

- Sequence type - counts for unique isomiR nucleotide sequences
- IsomiR type - sum of isomiR counts grouped by modification type
- miRNA type – sum of all isomiR counts by canonical miRNA ID

Results:

Our analysis of 36 samples derived from four different human primary cell types - skin fibroblasts (HDFs), microvascular endothelial cells from skin (HDMVECs), umbilical vein endothelial cells (HUVECs) and adipose derived mesenchymal stem cells (ASCs)- revealed high similarities in the miRNA and isomiR profiles of intracellular RNA and EV associated RNA. When comparing the number of distinct identified isomiRs to the number of mature miRNAs, we observed that in the cellular RNA fraction, there were between 3.2- to 3.8-fold more distinct isomiRs than mature miRNAs. Similarly, in the RNA isolated from EVs, between 3.1- to 3.6-fold more isomiRs were identified compared to mature miRNAs. In contrast, the protein-associated RNA fraction exhibited clear differences in the number of isomiR variants and length of miRNA mapping reads compared to intracellular miRNAs. In the protein fraction, only between 1.9- to 2.7-fold more isomiRs than mature miRNAs were detected.

Conclusion:

EVs preserve the integrity and diversity of the intracellular miRNA and isomiR profiles upon secretion, while protein-associated RNA shows evidence of selective degradation or modification, resulting in a distinct isomiR signature.

POSTER NUMBER: 77

Spike-in normalization enhances reliability of identifying CSF-enriched miRNAs by small RNA-sequencing

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Background:

Next Generation Sequencing has become the method of choice for genome-wide miRNA expression profiling. Normalization is a critical step in small RNA sequencing (sRNA-seq) data analysis to ensure accurate data interpretation and comparability across data sets. Conventional normalization methods rely on relative normalization, such as reads per million genome matching reads (RPM). Relative normalization methods assume that the composition of the small RNA transcriptome remains constant across different sample types. In cross-matrix comparisons this assumption can lead to the introduction of substantial bias resulting in flattened miRNA profiles, thereby masking true biological variation. Synthetic spike-ins, specifically designed to mimic endogenous miRNAs, have been proposed as an alternative normalization strategy to address these challenges.

Method:

In this study we compared donor-matched cerebrospinal (CSF) and serum samples to evaluate the effect of RNA complexity on different normalization strategies. The samples were processed using a standardized small RNA sequencing workflow, incorporating synthetic spike-ins. We compared the number of detected miRNAs and overall RNA complexity in relation to miRNA quantification and performance in unsupervised clustering and dimensionality reduction analyses between normalization methods.

Results:

Serum samples showed a higher complexity in RNA composition and number of distinct miRNAs compared to CSF samples. While RPM normalization failed to resolve differences in miRNA abundances between the sample matrices, spike-in normalization corrected for the inter-sample variability in RNA composition, enabling a clear separation of CSF and serum profiles in unsupervised clustering. Thus, our results demonstrate that the choice of normalization strategy significantly affects the sensitivity of miRNA quantification.

Conclusion:

Our findings highlight the critical influence of RNA complexity on normalization performance and demonstrate that spike-in normalization improves miRNA quantification accuracy and data comparability in sRNA-seq, particularly in studies involving heterogeneous sample types.

POSTER NUMBER: 78

Alterations of miRNA expression in metastatic Wilms' tumor

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Wilms' tumor (WT) is the most common renal malignancy in children and generally diagnosed before the age of five. Lungs are a major site of metastasis and recurrence while hepatic metastases are less common. Formalin-fixed, paraffin-embedded (FFPE) samples were used to investigate the alterations of microRNA (miRNA) expression in 10 primary and 6 metastatic WTs as well as in 10 healthy kidney specimens. Following RNA extraction and reverse transcription, samples were tested using a PCR array. In addition to 84 individual forward primers against specific miRNA targets, the arrays also contained primers against 4 endogenous controls. Studied miRNAs are known to play numerous roles in WT and/or other genitourinary tumors. Patterns of miRNA expression in metastatic WTs were compared to primary tumors and healthy kidney tissue by Student's t-tests and a Kruskal-Wallis test. Low p values were found in the case of five miRNAs (miR-17-3p, miR-34b-3p, miR-34c-5p, miR-375-3p, miR-616-3p), suggesting their possible involvement in the development of WT metastases.

POSTER NUMBER: 79

Differential impact of DGCR8 silencing on microRNA biogenesis in lymphoma cells

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MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional

level. Their maturation begins with the transcription of primary miRNAs (pri-miRNAs), which are processed by the Drosha-DGCR8 complex into precursor miRNAs (pre-miRNAs). These are then exported to the cytoplasm and further processed by Dicer into mature miRNAs. In this study, we investigated how DGCR8 knockdown affects miRNA biogenesis and lymphoma cell survival. Using lentiviral vectors carrying short hairpin RNAs (shDGCR8-1 and shDGCR8-2), we silenced DGCR8 in Hodgkin lymphoma cell lines (L1236, L540). Western blot analysis confirmed a significant reduction in DGCR8 protein levels upon knockdown. To assess changes in pri-miRNA levels, RNA sequencing was performed on L1236 cells transduced with shDGCR8-1 or a scrambled control (SCR). Our findings revealed that only 15 out of 767 pri-miRNAs showed increased expression upon DGCR8 inhibition. Quantitative RT-PCR validated the reduction of mature miR-155, miR-146a, and miR-17 levels, while their corresponding pri-miRNAs ie. pri-miR-155, pri-miR-146a and pri-miR-17~92) accumulated. Functionally, DGCR8 knockdown significantly reduced cell viability and induced apoptosis, with a threefold decrease in a number of viable L1236 cells after 10 days. Additionally, DGCR8-depleted cells exhibited increased sensitivity to ionizing radiation, with up to a fourfold increase in G1/G0 cell cycle arrest post 8 Gy irradiation. In conclusion, our results indicate that DGCR8 inhibition disrupts miRNA maturation in a miRNA-dependent manner and promotes apoptosis in lymphoma cells. Further research is ongoing to expand the analysis of pri-miRNA and mature miRNA dynamics following DGCR8 silencing.

POSTER NUMBER: 80

Transcription fidelity is needed for development and heat stress survival in Arabidopsis

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Transcriptional fidelity is vital to preserve the integrity of genetic information. Transcriptional fidelity maintenance is well characterised in yeast and metazoan, but remains understudied in plant kingdom. We employed genetic, molecular biology and deep sequencing tools to explore transcriptional fidelity, its regulation and its trans factors in *Arabidopsis thaliana*. Using circle-sequencing assay we analysed error landscape of the transcriptome at single nucleotide depth, and described the error features under ambient and heat stress conditions. We have found that the frequency of nucleotide mis-incorporations and insertions is significantly elevated under heat stress and have shown that RNA polymerase II associated elongation factor TFIIS ensures low error rates of transcription. The infidel mRNA transcripts containing insertions and deletions, which escape nuclear surveillance, are detected and degraded by the cytoplasmic nonsense-mediated decay mRNA quality control pathway. The clearance of infidel transcripts prevents the en masse production of faulty proteins to ensure proteostasis. We also provide evidence that nucleotide imbalance contributes to error-prone transcription.

In conclusion, the nuclear transcriptional fidelity factor TFIIS, and cytoplasmic mRNA and protein quality control systems cooperate to safeguard the correct genetic information flow and sustain development and adaptation to high temperature stress in plants.

POSTER NUMBER: 81

miR-652-3p target genes are involved in controlling of pro-inflammatory cytokines and autophagy in ischemic stroke

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Short, single-stranded RNA molecules called microRNAs (miRNAs) are pivotal in regulating gene expression post-transcriptionally, impacting a wide range of physiological and pathological processes. Numerous studies demonstrate an association between ischemic stroke—a leading cause of death and disability worldwide—and a number of microRNAs and their target genes. miRNAs, such as miR-652-3p, are involved in regulating inflammatory pathways, neuronal survival, and endothelial dysfunction.

This study investigates miR-652-3p and its target genes' roles in ischemic stroke pathways. We used the miRDB and Diana-microT 2023 miRNA target prediction tools to identify miR-652-3p targets with a target score above 80 (miRDB) and interaction score 0.8 (Diana-microT 2023). We then used ShinyGo 0.82 for pathway enrichment analysis to find significant associations (minimal pathway length 10) between miR-652-3p target genes and related proteins and pathways.

Our results confirm miR-652-3p's targets importance in neuronal development and angiogenesis pathways, such as axon development, neuron projection, and VEGF production and regulation. But also show miR's involvement in regulation of inflammatory processes including the control of pro-inflammatory cytokines and cell signaling through IL-6, IL-12, IL-1 and interferon gamma production. Such findings suggest that miR-652-3p may prevent secondary damage of neurons such as ischemic reperfusion injury through negative regulation of autophagy and brain edema.

In summary, our findings suggest miR-652-3p plays a significant role in ischemic stroke and mitigating reperfusion damage. miR-652-3p and its predicted targets seemed to be promising in future diagnostic and therapeutic strategies aimed at reducing the consequences of cerebral ischemia.

POSTER NUMBER: 82

Circadian expression and time-of-day interacting partners of poly(A)-specific ribonuclease

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The poly(A) tail determines mRNA stability, translatability and degradation. Its length varies throughout mRNA lifespan due to tail-elongating or tail-shortening enzyme activities that affect translational efficiency. Studies in mouse liver revealed that changes in poly(A) tail length are under the control of the circadian clock, and even transcripts that are not rhythmically transcribed may be rhythmically destabilized and degraded, resulting in circadian patterns of protein production. The shortening of the poly(A) tail is the first and rate-limiting step of eukaryotic mRNA degradation and is catalyzed by deadenylases, yet their role in circadian gene regulation remains poorly understood. To date, AtHESPERIN is the only deadenylase following circadian expression in plants. A key question is whether deadenylases are continuously synthesized throughout the solar day and are recruited as needed by critical protein partners on selected targets, or their expression is rhythmic, aligning with the turnover of circadian RNAs based on temporal co-occurrence.

Here, we focus on poly(A)-specific ribonuclease (PARN), a well-characterized mammalian deadenylase involved in both mRNA turnover and the maturation of non-coding RNAs, and we study its expression in synchronized human cells and mice maintained under standard light/dark cycles. We demonstrate that PARN exhibits circadian mRNA and protein levels in HEK293T cells and mice liver, an organ with well-established rhythmicity. To uncover the molecular mechanisms regulating its function, we performed large-scale proteomics to define PARN's interactome under normal and disrupted photoperiods.

Our findings reveal time-of-day-dependent interaction dynamics, suggesting that PARN's activity and localization are modulated by circadian rhythms. We identify novel protein partners linking PARN to previously unexplored cellular processes, including autophagy and P-bodies. These results suggest that PARN may be temporally redirected to targets among different functional pathways in response to the changing needs of the cell across the circadian cycle.

POSTER NUMBER: 83

Distinguishing diabetic ischemic stroke: The role of serum exosomal miR-652-3p

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The global prevalence of both diabetes and stroke is rising, with diabetes significantly increasing the risk of stroke through various molecular pathways. Numerous microRNAs are implicated in both conditions and may serve as potential biomarkers. This study sought to identify miRNAs that distinguish between diabetic and non-diabetic patients following reperfusion therapy.

Seventy-two patients (25 with diabetes, 47 without) provided blood samples on day one (acute stroke phase) and day ten post-reperfusion therapy. Exosomal miRNAs were extracted from serum samples and their quantities measured using RT-qPCR. We normalized the results to miR-320a. The difference in fold change (FC) between day 10 and the acute stroke phase was analyzed for diabetic and non-diabetic patients. Subsequently, miRNA data from the NCBI's GSE262614 study were analyzed to compare levels between diabetic and non-diabetic, non-stroke populations.

Our research showed significant difference in miR-652-3p levels ($p=0.048$, Student's t-test) between diabetic and non-diabetic patients with calculated moderate effect (g Hedges= 0,49; d Cohen = 0.49). In silico analysis of NCBI's GSE262614 dataset suggests that, while miR-652-3p is associated with insulin sensitivity, its expression levels are not significantly different in diabetic patients compared to healthy controls.

Given the homogeneity of the study groups across modified Rankin Scale score, age, and ischemia severity, microRNA expression differences may reliably differentiate diabetic from non-diabetic stroke patients, irrespective of stroke onset time.

Findings show miR-652-3p may be useful in distinguishing diabetic and non-diabetic patients treated with reperfusion therapy.

POSTER NUMBER: 84

The door opens only for those who have already walked through it: mechanistic dissection of the parent-of-origin effect in the toxin-antidote element *slow-1/grow-1*

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Toxin-antidote elements (TAs) are parasitic genetic elements that increase their frequency in populations by poisoning individuals that do not inherit at least one copy. We recently discovered that the *slow-1/grow-1* TA from *Caenorhabditis tropicalis* is specifically inactive when paternally inherited. This parent-of-origin effect stems from transcriptional repression of the *slow-1* toxin by the PIWI-interacting RNA (piRNA) defense pathway. Remarkably, when *slow-1/grow-1* is maternally inherited, *slow-1* repression is halted by a translation-independent role of its maternal mRNA. That is, *slow-1* transcripts loaded into eggs—but not SLOW-1 protein—are necessary and sufficient to counteract piRNA-mediated repression of zygotic *slow-1* in the germline of the embryo. However, although sequence identity between maternal and zygotic transcripts is clearly critical, the molecular basis of this phenomenon—epigenetic licensing—remains unclear. Because maternal expression of the reporter mScarlet is sufficient to license zygotic mScarlet::*slow-1*, we leveraged this assay to decouple and systematically modify maternal and zygotic genes and define the rules of licensing. Our results strongly suggest that the licensing signal is robust to variation in maternal dosage and that sequence matching between the maternal transcript and either the 5' or 3' of the zygotic transcript is sufficient for licensing. However, we found that licensing is highly sensitive to the length of the matching region: maternal transcripts containing only 15% or 50% of the mScarlet coding sequence (with constant overall length) failed to license. We are now testing whether the 5' or 3' of the maternal transcript are equally effective in licensing, the minimum percent identity required for it, and the generality of these principles across other loci. We anticipate that this framework will help us elucidate how nematodes—and potentially other species—discriminate self from non-self transcripts in the germline.

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