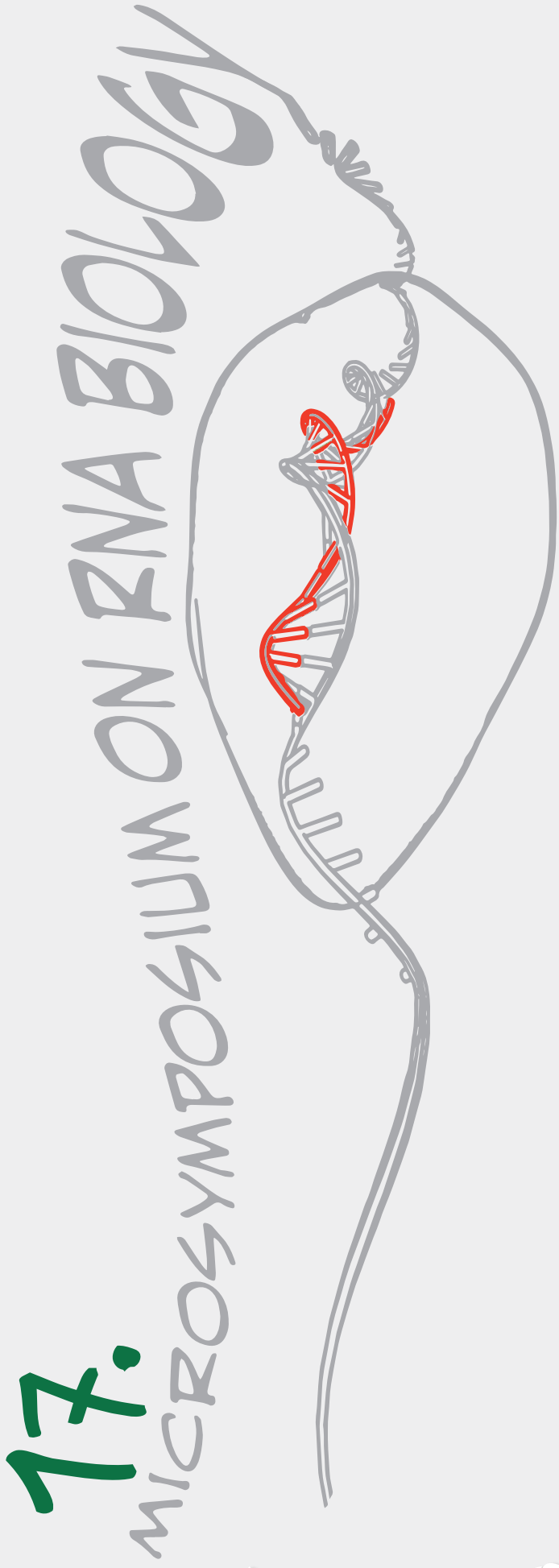

17th Microsymposium on RNA Biology

Vienna BioCenter, May 3rd - 5th 2023

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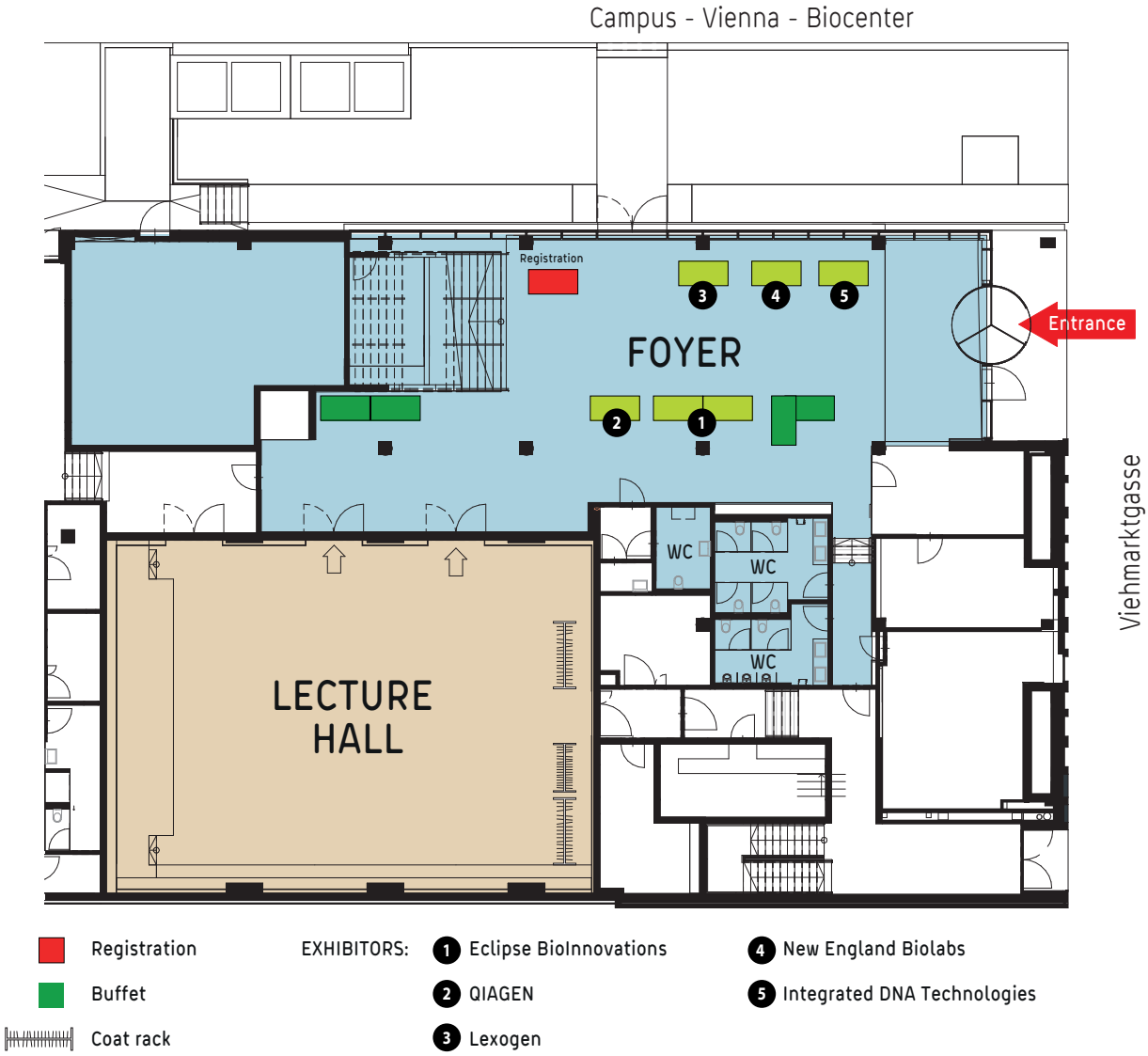

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Buffet

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17th MICROSYMPOSIUM on RNA Biology

Wednesday, May 03

11:00 **welcome & introduction**

Session 1: Transcriptional control

Chair: Julius Brennecke

11:15 **Irinia Solovei** (Ludwig Maximilian University of Munich)
Transcription shapes spatial organization of eukaryotic genes

11:45 **Olivier Duss** (European Molecular Biology Laboratory)
Tracking assembly and function of single active RNPs in real-time

12:15 **Max Wilkinson** (Broad Institute of MIT and Harvard)
Structure of the R2 non-LTR retrotransposon initiating target-primed reverse transcription

12:35 **Joanna Jachowicz** (Institute of Molecular Biotechnology Austria)
Short stories about long RNAs

13:05 *lunch break*

Session 2: RNA processing (pt. 1)

Chair: Sebastian Falk

14:05 **Niels Gehring** (University of Cologne)
Two-factor authentication and fingerprints: how cells detect defective mRNAs

14:35 **Irene Beusch** (University of California, San Francisco)
Functional genomics of the human spliceosome in haploid cells reveals in vivo functions of SUGP1

14:55 *coffee break*

15:25 **Florian Heyd** (Freie Universität Berlin)
Body temperature-controlled alternative splicing: from basic to translational research

15:55 **Daria Riabov Bassat** (Research Institute of Molecular Pathology)
Structure of the recycling human U5 snRNP

16:15 *coffee break & group picture*

16:45 **PhD workshop pt.1**

Tsimafei Navalayeu (Max Perutz Labs)

CRISPR/Cas9 screening resolves the functions, cellular assembly and proteostatic control of mammalian RNA exosome subunits

Young Yoon Lee (Seoul National University)

Molecular and structural insights into small RNA production by human DICER

David Steinbrecht (Charité)

Modelling how mRNA flows through subcellular compartments

17:30 *coffee break*

Session 2: RNA processing (pt. 2)

Chair: Javier Martinez

18:00 **Stefanie Jonas** (ETH Zurich) [EMBO Young Investigator Lecture]

Basis of gene-specific transcription attenuation by the Integrator complex

18:30 **Torben Heick-Jensen** (Aarhus University)

Nuclear Sorting of RNA

19:00 **Niko Amin-Wetzel** (Institute of Science and Technology Austria)

The macoilin-CCR4-Not complex at the endoplasmic reticulum

19:20 *Symposium dinner & informal poster session*

Thursday, May 04

Session 3: RNA transport

Chair: Clemens Plaschka

09:00 **Karsten Weis** (ETH Zurich)
The role of DEAD-box ATPases in the regulation of ribonucleoprotein complex condensation

09:30 **Marlene Oeffinger** (Montreal Clinical Research Institute)
Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in S.cerevisae

10:00 **Alexa McIntyre** (University of Zurich)
Phosphorylation controls mRNA retention via nuclear speckle cohesion during stress

10:20 coffee break

Session 4: Development

Chair: Andrea Pauli

10:50 **Geraldine Seydoux** (Johns Hopkins University)
Regulation of RNA granule assembly and function

11:20 **Nadine Vastenhouw** (University of Lausanne)
The spark of life. Initiating transcription in embryos.

11:50 break

12:10 **Valérie Hilgers** (Max Planck Institute of Immunobiology and Epigenetics)
Non-coding RNAs drive cytoplasmic compartmentalization and neuronal function

12:40 **Laura Arribas-Hernandez** (University of Copenhagen)
Growth control by an m6A/YTHDF axis in plants

13:00 lunch break

14:00 **PhD workshop pt.2**

Jasper van Lopik (University of Cambridge)

Unistrand piRNA clusters are an evolutionary conserved mechanism to suppress endogenous retroviruses across the Drosophila genus

Klara Kuret (National Institute of Chemistry)

3'UTR mRNP repositioning triggers selective mRNA decay to drive rapid embryonic morphogenesis

Marcos Iuri Roos Kulmann (Institute of Molecular Genetics)

Activation of antiviral RNAi in mammals

Franziska Hoerbst (John Innes Centre)

True or false, heads or tails, SNP or error, mobile or not; And the implication for determining the mobility of mRNA

15:00 *Poster session I*

16:30 **PhD workshop pt.3**

Peter Wang (Whitehead Institute)

Guide-RNA sequence determinants of Argonaute2 slicing kinetics and conformational dynamics

Ivan Milenkovic (Centre for Genomic Regulation)

Epitranscriptomic fingerprinting: tissue and cell type deconvolution from rRNA modification patterns

Daniel Buendia Avila (Gregor Mendel Institute for Molecular Plant Biology)

Everything Everywhere All at once? Understanding transposon silencing in the minute flowering plant Wolffia

17:15 *end of meetings*

Free evening for attendees

Friday, May 05

Session 5: Small RNAs

Chair: Stefan Ameres

09:00 **Joshua Mendell** (UT Southwestern Medical Center)
New functions and regulators of noncoding RNAs in mammals

09:30 **Virginia Busetto** (Max Perutz Labs / University of Vienna)
MUT-7: a conserved exoribonuclease involved in sRNA biogenesis

09:50 coffee break

10:20 **Felipe Teixeira** (University of Cambridge) [EMBO Young Investigator Lecture]
Transposable element regulation and DNA damage response during germline development

10:50 **Katarzyna Kuduk** (Eclipse Bioinnovations) [sponosred talk]
Eclipse Bioinnovations: Accelerating the RNA genomics discoveries

11:05 **Ramesh Pillai** (University of Geneva)
RNA modifications in control of mammalian gene expression

11:35 Poster session II

13:00 lunch break

Session 6: Translation control

Chair: Arturo Mari-Ordonez

14:00 **Yiliang Ding** (John Innes Centre)
RNA structure, a hidden regulator in living cells

14:30 **Milan Gerovac** (Institut für Molekulare Infektionsbiologie)
Immediate targeting of host ribosomes by jumbo phage encoded proteins

14:50 **Axel Innis** (Institut Européen de Chimie et Biologie)
The ribosome: when RNA meets protein to sense small molecules

15:20 **PhD award & closing remarks**

15:30 Light bites & socializing

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SPEAKER ABSTRACTS

Session 1: Translational control

Transcription shapes spatial organization of eukaryotic genes
Irinia Solovei
Ludwig Maximilian University of Munich

Despite the well established role of nuclear organization in the regulation of gene expression, little is known about the reverse: how transcription shapes the spatial organization of the genome. In particular, given the relatively small sizes of genes and the limited resolution of light microscopy, the structure and spatial arrangement of a single transcribed gene are still poorly understood. We made use of several long highly expressed mammalian genes and demonstrated that they form Transcription Loops with polymerases moving along the loops and carrying nascent RNAs that undergo co-transcriptional splicing. This finding rules out a popular hypothesis about eukaryotic transcription occurring in so called Transcription Factories with immobilized polymerases and genes reeling through them. Transcription loops dynamically modify their harboring loci and extend into the nuclear interior suggesting an intrinsic stiffness of these structures. Both experimental evidence and polymer modeling support the hypothesis that transcription loop stiffness is caused by the dense decoration of transcribed genes with multiple voluminous nascent RNPs. We propose that transcription loop formation is the universal principle of eukaryotic gene expression that has not been appreciated until now due to the limitation of light microscopy resolution and due to the small size or low expression of studied genes.

Tracking assembly and function of single active RNPs in real-time
Olivier Duss
European Molecular Biology Laboratory

Assembly of protein-RNA complexes (RNPs) is a most fundamental process in all life forms. Whereas in cells many such complexes form on the nascent RNA while it is emerging from the RNA polymerase and in coordination with many other cellular factors and processes, our current understanding is based merely on the interaction of pre-formed protein and RNA molecules but we lack quantitative molecular understanding on how multiple different processes in RNP assembly cooperate and are regulated and how different molecular machines interact. To obtain more quantitative models of how RNPs assemble and work in context, we combine novel cutting-edge in vitro multi-color single-molecule approaches, structural biology, biochemical assays and in vivo experiments. In the first part, I will present our current work on understanding how transcription is coupled with translation in bacteria. We have reconstituted in vitro the complete active transcription-translation coupling system and developed multicolor single-molecule fluorescence microscopy assays to track in real-time how single RNA molecules are actively transcribed (Duss et al., Cell, 2019) and simultaneously translated by the ribosome. Furthermore, we are detecting the direct physical interaction between both machineries. Our experiments show, for example, how the ribosome slows down upon colliding into the RNA polymerase or that stochastic coupling/uncoupling occurs also if the two machineries are separated by >100nt. In the second part, we will look into the dynamic process of how RNA is modified to m6A by the human Mettl3/14 enzyme and then dynamically recognized by the YTHDC1 reader protein. Simultaneously detecting both specific Mettl3/14 and YTHDC1 binding to the same target RNA molecule, we see that upon modification, the transient and mostly unproductive Mettl3/14 binding events are replaced by a dynamic YTHDC1 binding pattern suggesting a dynamic m6A-mediated interactome switch. Overall, by tracking the conformation and interaction dynamics of different macromolecular machines over time, our methods allow us to investigate how different macromolecular machines cooperate to fine-tune gene expression.

Structure of the R2 non-LTR retrotransposon initiating target-primed reverse transcription
Max Wilkinson
Broad Institute of MIT and Harvard

Non-LTR retrotransposons, or Long Interspersed Nuclear Elements (LINEs), are an abundant class of eukaryotic transposons that insert into genomes by target-primed reverse transcription (TPRT). During TPRT, a target DNA sequence is nicked and primes reverse transcription of the retrotransposon RNA. Structural information is lacking for this entire class of mobile genetic elements. Here, we report the cryo-electron microscopy structure of the Bombyx mori R2 non-LTR retrotransposon initiating TPRT at its ribosomal DNA target. The target DNA sequence is unwound at the insertion site and recognized by an upstream motif. An extension of the reverse transcriptase (RT) domain recognizes the retrotransposon RNA and guides the 3' end into the RT active site to template reverse transcription. We used Cas9 to retarget R2 in vitro to non-native sequences, suggesting future use as a reprogrammable RNA- based gene-insertion tool.

Short stories about long RNAs
Joanna Jachowicz
Institute of Molecular Biotechnology Austria

Abstract: Although thousands of lncRNAs are encoded in mammalian genomes, their mechanisms of action are largely uncharacterized because they are often expressed at significantly lower levels than their proposed targets. One such lncRNA Xist mediates chromosome-wide gene silencing on one of the two X chromosomes to achieve gene expression balance between males and females. However, how a limited number of Xist molecules can mediate robust silencing of a significantly larger number of target genes (~1 Xist RNA: 10 gene targets) while maintaining specificity exclusively to genes on the X within each cell, is unclear. I will present our recent observations uncovering a spatial amplification mechanism that allows Xist to achieve these two essential but countervailing regulatory objectives: chromosome-wide gene silencing and specificity to the X. I will also discuss how this spatial amplification mechanism may be a more general mechanism by which other low abundance lncRNAs can balance specificity to, and robust control of, their regulatory targets.

Session 2: RNA processing (pt. 1)

Two-factor authentication and fingerprints: how cells detect defective mRNAs
Niels Gehring
University of Cologne

Nonsense-mediated mRNA decay (NMD) is a conserved surveillance pathway targets mRNAs that contain premature termination codons (PTCs). By selectively degrading these PTC-containing mRNAs, NMD helps to maintain proper gene expression and prevent the accumulation of potentially harmful truncated proteins. NMD is a complex process that involves a network of proteins interacting with each other and forming NMD-promoting complexes on the target mRNA. The detection of PTCs requires the presence of the exon junction complex (EJC), which is deposited on the mRNA during splicing. EJCs are removed during translation from mRNAs with a full-length open reading frame, but remain bound on the mRNA when they are located sufficiently downstream of a PTC. In this case, the EJC serves as mark (=fingerprint) that is recognized by the NMD machinery and leads to the activation of the degradation phase of NMD. According to current models, the degradation of NMD targets involves two main branches. The first branch depends on phosphorylated UPF1 interacting with SMG5-SMG7, which then recruits the CCR4-NOT deadenylation complex to promote mRNA deadenylation, decapping, and exonucleolytic degradation. The second branch involves the endonuclease SMG6 cleaving the NMD-targeted transcript near the NMD-activating stop codon. SMG5-SMG7 and SMG6-mediated degradation are both considered redundant and independent because they target the same transcripts, but downregulation of individual factors only partially inhibits NMD. We found recently that the combined loss of SMG5-SMG7 efficiently inactivates NMD, and SMG6 was catalytically inactive in cells depleted of SMG5-SMG7. We propose an improved model, in which two authentication steps are required for the execution of NMD. During the first step, UPF1 becomes (hyper)phosphorylated by SMG1 as consequence of an NMD-activating mRNP composition around the PTC. As a second step, SMG5 and SMG7 recruitment to hyper-phosphorylated UPF1 license SMG6-mediated degradation of the target transcript. This two-factor authentication prevents untimely endonucleolytic decay and restricts the activity of SMG6 to authentic NMD targets. Recent work in the lab concentrated on the first authentication step and how the SMG8 and SMG9 proteins regulate the function of SMG1. I will present the recent findings and discuss their implications for the NMD process.

Functional genomics of the human spliceosome in haploid cells reveals in vivo functions of SUGP1
Irene Beusch
University of California, San Francisco

Most of our understanding of how the spliceosome operates in vivo comes from studies of mutants in yeasts, but human splicing displays major differences. However, applying genetics to understand the spliceosomal basis for these differences is challenging. To identify informative alleles, we implemented CRISPR-Cas9 base editing in haploid human cells (eHAPs), producing a library that generates point mutations in thousands of individual residues in over 150 spliceosomal proteins. We tested this approach by performing a screen to identify mutants resistant or hypersensitive to pladienolide B (plaB), an inhibitor of SF3b in the U2 snRNP. Hypersensitive mutants occurred in SF3a and SF3b subunits and other A complex components but also in factors thought to act only later in splicing. Resistant mutations showed anticipated sequence changes in SF3b subunits near the plaB binding pocket. Viable resistance substitutions map not only to the pladienolide B binding site but also to the G-patch (ATPase activator) domain of SUGP1, which lacks orthologs in yeast. We used these mutants and biochemical approaches to identify the spliceosomal disassemblase DHX15/hPrp43 as the ATPase ligand for SUGP1. These and other data support a model in which SUGP1 promotes splicing fidelity by triggering early spliceosome disassembly in response to kinetic blocks.The ability to identify functional significant alleles of human spliceosomal proteins in vivo opens up exciting avenues to the study of their functions in a manner not previously possible. Our approach also provides a template for the analysis of other essential cellular machines.

Body temperature-controlled alternative splicing: from basic to translational research
Florian Heyd
Freie Universität Berlin

In recent work, we have uncovered mechanistic details how subtle changes in body temperature globally control alternative splicing and gene expression. We have characterized a family of kinases, CLKs, whose activity responds extremely sensitive to changes in the physiologically relevant temperature range. CLKs phosphorylate SR proteins and the phosphorylation status of SR proteins then controls all steps of pre-mRNA processing. In particular, we found that alternative splicing leading to the generation of premature termination codons is highly temperature sensitive, thereby controlling expression levels of the respective transcripts by directing them to the nonsense-mediated decay pathway. We therefore suggest that alternative splicing-coupled to nonsense-mediated decay (AS-NMD) plays a major role in establishing the transcriptome in response to changes in small changes in body temperature. Interestingly, manipulation of body temperature has been used in clinical settings, where hypothermia has been shown to be neuroprotective, whereas hyperthermia is used in addition to chemotherapy in the treatment of some tumors. However, the molecular mechanisms that connect changes in body temperature with beneficial effects of thermotherapies have remained largely enigmatic. We have started to investigate several temperature sensitive AS-NMD events that control the expression of key genes in neuroprotection and tumor thermotherapy. In one example, we have characterized a temperature-controlled AS-NMD event that is solely responsible for cold-induced RBM3 expression. RBM3 has been shown to act neuroprotective in diverse settings and its cold-induced expression has been suggested to (at least partially) mediate the neuroprotective effect of hypothermia. We have furthermore developed antisense oligonucleotides (ASOs) that are highly effective in controlling this AS-NMD event, leading to increased RBM3 expression and neuroprotection at normothermia. We suggest that this experimental set up provides a blueprint for the characterization of additional disease-relevant temperature-controlled AS-NMD events and the design of ASOs that may be used in translational applications.

Structure of the recycling human U5 snRNP
Daria Riabov Bassat
Research Institute of Molecular Pathology

The spliceosome is a multi-megadalton molecular machine that catalyses excision of introns from pre-mRNA. Splicing requires that the spliceosome assembles anew on each intron from five small nuclear ribonucleoprotein particles (snRNPs; U1, U2, U4, U5 and U6) which are recycled after splicing termination to regenerate the components for subsequent splicing rounds. Among the snRNPs, the U5 snRNP forms the 'heart' of the spliceosome, chaperones the formation of the RNA active site, and is substantiality remodeled during splicing. Despite much progress in the structural understanding of pre-mRNA splicing [1-3], how the U5 snRNP is recycled for new splicing rounds remains poorly understood.
To address this gap, we used cryo-electron microscopy (Cryo-EM) to determine the structure of the recycling human U5 snRNP. The structure reveals how the transient U5 snRNP protein CD2BP2 acts at several sites within the U5 snRNP to prepare it for the controlled and ATP-independent joining with the U4/U6 di-snRNP to form the U4/U6.U5 tri-snRNP, the largest building block needed for new spliceosome assembly. Specifically, we show how CD2BP2 may (1) re-position domains of PRP8, (2) control the binding of PRP6 and TNXL4A, and (3) facilitate its own release through an ATP-independent mechanism. Taken together, our data show the structure of an isolated and recycling U5 snRNP and suggest mechanisms by which CD2BP2 primes the U5 snRNP for tri-snRNP assembly.
1. Kastner B, Will CL, Stark H, Lührmann R. Structural Insights into Nuclear pre-mRNA Splicing in Higher Eukaryotes. Cold Spring Harb Perspect Biol. 2019 Nov 1;11(11):a032417. doi: 10.1101/cshperspect.a032417. PMID: 30765414; PMCID: PMC6824238.
2. Plaschka C, Newman AJ, Nagai K. Structural Basis of Nuclear pre-mRNA Splicing: Lessons from Yeast. Cold Spring Harb Perspect Biol. 2019 May 1;11(5):a032391. doi: 10.1101/cshperspect.a032391. PMID: 30765413; PMCID: PMC6496352.
3. Wan R, Bai R, Zhan X, Shi Y. How Is Precursor Messenger RNA Spliced by the Spliceosome? Annu Rev Biochem. 2020 Jun 20;89:333-358. doi: 10.1146/annurev-biochem-013118-111024. Epub 2019 Dec 9. PMID: 31815536.

PhD workshop pt.1

CRISPR/Cas9 screening resolves the functions, cellular assembly and proteostatic control of mammalian RNA exosome subunits

Tsimafei Navalayeu
Max Perutz Labs

The RNA exosome is an evolutionary conserved, multi-subunit 3'-to-5'exoribonuclease complex required for the processing and/or degradation of various types of RNA substrates. While the structural basis for exosome-mediated RNA decay and many of its cellular substrates have been extensively studied, a comprehensive view on the physiological and molecular function of individual core complex components as well as their cellular assembly and proteostatic control remains poorly understood.Here, we established a doxycycline-inducible dual guide CRISPR/Cas9 approach in mouse embryonic stem cells (mESCs) that enables the systematic parallel depletion of essential gene products at timescales that expose direct molecular and phenotypic consequences. By applying this experimental strategy to individual components of the RNA exosome and combining it with cell growth competition assays, we confirmed the essential function of most complex constituents except for the peripheral component Exosc1. Northern blot and qPCR experiments revealed that Exosc1 is needed for rRNA processing, but partially dispensable for the degradation of other nuclear RNA exosome targets (i.e. PROMPTs). Notably, depletion of individual exosome subunits resulted in the selective proteolytic degradation of specific complex components by the ubiquitin-proteasome system. Based on this observation, we performed systematic quantitative Western blot and IP-MS analyses to delineate the cellular hierarchy of RNA exosome complex assembly. Our observations strongly support a model, where initial formation of a core-trimer consisting of Exosc2, Exosc4 and Exosc7 is followed by the sequential recruitment of Exosc6, Exosc8, Exosc9, Exosc5 and Exosc3 in a directed order. The assembly of an octameric core (without Exosc1) is required to stabilize the cytoplasmic catalytic component Dis3L but not its nuclear counterpart Dis3. Consistent with its phenotypically dispensable role, Exosc1 it is the last component of RNA exosome core assembly and is required to stabilize the nuclear exoribonuclease Exosc10. In summary, we established a novel experimental strategy for time-controlled loss-of-function studies of essential genes in mESCs, establishing Exosc1 as a partially dispensable and peripheral component of the otherwise essential RNA exosome core complex and revealing unprecedented insights into the cellular assembly and proteostatic control of the RNA exosome.

Molecular and structural insights into small RNA production by human DICER

Young Yoon Lee
Seoul National University

RNA silencing relies on specific and efficient processing of double-stranded RNA (dsRNA) by Dicer, which yields microRNAs (miRNAs) and small interfering RNAs (siRNAs). However, our current knowledge of Dicer's specificity is limited to secondary structures of its substrates: a dsRNA of ~22 bp with a 2-nt 3' overhang and a terminal loop. We here found evidence pointing to an additional sequence-dependent determinant beyond these structural properties. To systematically interrogate features of precursor miRNAs (pre-miRNAs), we carried out massively parallel assays with pre-miRNA variants and human DICER. Our analyses revealed a deeply conserved cis-acting element, termed the 'GYM' motif (paired G, paired pyrimidine, and mismatched C or A) near the cleavage site. GYM motif promotes processing at a specific position and can override the previously identified 'ruler'-like counting mechanisms. Consistently, integrating this motif into short hairpin RNA (shRNA) or Dicer substrate siRNA (DsiRNA) potentiates RNA interference. This study uncovers an ancient principle of substrate recognition by metazoan Dicer and implicates its potential in RNA therapeutics design. Yet, our understanding of pre-miRNA processing is still limited due to lack of structure of DICER in a catalytic state. Here we determined the first 'active-state' structure of DICER bound to pre-miRNA. DICER undergoes large conformational changes to achieve active state. dsRNA binding domain (dsRBD) anchors pre-miRNA in a specific position through sequence-specific recognition of the 'GYM motif'. Our structure unveils a previously uncharacterized configuration of 5' end of pre-miRNA inserted into a basic pocket. In this pocket, a group of arginines recognize 5' terminal base (disfavoring guanine), explaining hDICER's specificity and cleavage site determination. We identify cancer-associated mutations in GYM motif- and terminal base-recognizing residues, which impair DICER activity in vitro and miRNA biogenesis in cells. Our study explains how DICER recognizes pre-miRNAs with stringent specificity and allows a mechanistic understanding of hDICER-related diseases.

Modelling how mRNA flows through subcellular compartments
David Steinbrecht
Charité

During their life cycle, eukaryotic mRNAs are transcribed and processed in the nucleus and then exported to the cytoplasm. Next, they are targeted to distinct subcellular compartments where they can execute their functions until they are finally degraded. The intracellular dynamics of RNA transcription, processing and localisation remain poorly characterised. Yet, obtaining the quantitative temporal and spatial information about each step of the RNA metabolism is of fundamental interest for global understanding of transcriptome regulation and cell homeostasis. In this ongoing study, we quantify mRNA flow rates between subcellular compartments in mouse embryonic stem cells. Combining standard sequencing methods and biochemical cell fractionation with metabolic labelling and mathematical modelling we were able to determine the kinetic rates of mRNA transcription, splicing, nuclear export, and cytosolic and membrane stability for more than 7000 mRNAs. Overall, our data suggests nuclear export to be the rate limiting step for most of the transcripts. Genes encoding transcription factors or immediate early gene possess fast kinetic rates. Differentially localized mRNAs exhibit distinct combinations of rate constants, suggesting modular control within subcellular compartments. We show cytosolic stability is generally high for cytosol-localized mRNA and that membrane stability is high for membrane-localized mRNA. Proteins containing transmembrane domains or signal peptides have low cytoplasmic stability and high membrane stability. Nuclear-encoded mitochondrial proteins have slow nuclear export and otherwise similar features of nucleocytoplasmic kinetics that do not resemble co-translational targeting to the mitochondria. Altogether, our results are a valuable resource for spatiotemporal information of gene expression regulation in mammalian cells.

Session 2: RNA processing (pt. 2)

Basis of gene-specific transcription attenuation by the Integrator complex
Stefanie Jonas
ETH Zurich

Integrator (INT) is a multi-subunit modular RNA processing complex, which exhibits both RNA endonuclease and protein phosphatase activity. It is responsible for transcription termination at the 3' ends of a diverse array of RNA polymerase II (RNAP2) transcribed non-coding RNAs, as well as for transcription attenuation at a large number of protein coding genes. There, it inhibits the synthesis of full-length mRNAs by terminating RNAP2 at promoter-proximal pausing sites. This INT-mediated tapering of gene expression by premature termination of transcription occurs predominantly at stimulus responsive genes and is essential for cell differentiation. Although INT mediates transcription termination at varying classes of RNAs in diverse biological contexts, how it achieves specificity in a gene- and context-dependent manner has remained elusive. Using a combination of proteomics, interaction studies and structural characterization, we identified a diverse set of transcription factors (TFs) that associate directly with defined surfaces on INT. Stress conditions lead to changes in the types of TFs bound by INT, and quantitative binding studies suggest that TF affinities can be modulated by post-translational modifications. Integrated multi-omics data show that INT and its TF interactors regulate significantly overlapping sets of genes and indicate that these TFs recruit INT to specific genomic loci. Consistently, cellular stress response depends on intact TF-INT binding. Taken together, our data suggest that TFs lend INT specificity to elicit targeted attenuation as a transcriptional response in defined biological contexts

Nuclear Sorting of RNA
Torben Heick-Jensen
Aarhus University

Mammalian genomes are promiscuously transcribed, demanding efficient nuclear sorting of RNA. On the degradative side of the sorting process, the RNA exosome is a central ribonuclease. In the nucleoplasm, it is assisted by its adaptors the Nuclear EXosome Targeting (NEXT) complex and the PolyA eXosome Targeting (PAXT) connection. Via its association with the ARS2 and ZC3H18 proteins, NEXT/exosome is recruited to capped and short unadenylated transcripts. Instead, PAXT was considered to provide exosome access to longer and adenylated substrates. Through mutational analysis of the core PAXT component ZFC3H1, a separate branch of the PAXT pathway is uncovered, which targets short adenylated RNAs and relies on a direct ARS2-ZFC3H1 interaction. Further, similar acidic short linear motifs (SLiMs) of ZFC3H1 and ZC3H18 are demonstrated to compete for binding a common basic patch of ARS2. Consequently, while promoting NEXT function, ZC3H18 antagonizes PAXT activity. This unprecedented organization of RNA decay complexes provides for the concerted activation of NEXT and PAXT pathways. Conspiciously, the basic patch of ARS2 also binds an acidic SLiM of the transcription restriction factor ZC3H4. This interaction recruits the ZC3H4/WDR82 dimer to chromatin to elicit RNAPII termination, independent of other early termination pathways defined by the cleavage and polyadenylation (CPA) and Integrator (INT) complexes. ZC3H4 in turn directly connects to the ZCCHC8 component of the NEXT complex, hereby facilitating rapid degradation of the nascent RNA. Finally, ARS2 also interacts with SLiM-containing RNA maturation factors, like FLASH and PHAX. This positions ARS2 centrally in the nuclear RNA sorting process, the putative mechanism of which will be discussed.

The macoilin-CCR4-Not complex at the endoplasmic reticulum

Niko Amin-Wetzel
Institute of Science and Technology Austria

The endoplasmic reticulum (ER) is the major site of synthesis for secreted and endomembrane proteins and is thought to account for a third of a cell's translational activity. While a great deal is known about the regulation of recruitment and translation of mRNAs at the ER, little is known about the subsequent fate of these mRNA molecules. We have discovered that the ER transmembrane protein macoilin interacts with the CCR4-Not complex at the ER. The CCR4-Not complex is conserved across eukaryotes and is involved in many aspects of gene expression regulation. Notably, it regulates mRNA degradation and translation through its deadenylation activity. Macoilin is conserved throughout Metazoa and is highly expressed in neurons. Our proximity labeling data suggest that the macoilin::CCR4-Not interaction is conserved from mammals to C. elegans. Little is known about the molecular function of macoilin, but C. elegans macoilin mutants show defects in multiple behaviors including responses to oxygen, temperature, and pheromones. Though overall healthy, C. elegans lacking macoilin exhibit defective neuronal calcium signaling responses to oxygen and temperature stimuli. Our mRNA tethering assays show that mRNAs localized to macoilin are degraded leading to decreased gene expression. Together our data point towards a conserved mRNA regulatory complex at the ER involving macoilin and the CCR4-Not complex with a particular importance in neurons.

Session 3: RNA transport

The role of DEAD-box ATPases in the regulation of ribonucleoprotein complex condensation
Karsten Weis
ETH Zurich

Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in S.cerevisae
Marlene Oeffinger
Montreal Clinical Research Institute

To determine which transcripts should reach the cytoplasm for translation, eukaryotic cells have established mechanisms to regulate selective mRNA export through the nuclear pore complex (NPC). The nuclear basket, a substructure of the NPC protruding into the nucleoplasm, is thought to function as a stable platform where mRNA-protein complexes (mRNPs) are rearranged and undergo quality control prior to export, ensuring that only mature mRNAs reach the cytoplasm. Here, we use proteomic, genetic, live-cell, and single-molecule resolution microscopy approaches in budding yeast to demonstrate that basket formation is dependent on RNA polymerase II transcription and subsequent mRNP processing. We further show that while all NPCs can bind Mlp1, baskets assemble only on a subset of nucleoplasmic NPCs, and these basket-containing NPCs asso- ciate a distinct protein and RNA interactome. Taken together, our data point toward NPC heterogeneity and an RNA-dependent mechanism for functionalization of NPCs in budding yeast through nuclear basket assembly.

Phosphorylation controls mRNA retention via nuclear speckle cohesion during stress
Alexa McIntyre
University of Zurich

Nuclear speckles are membraneless organelles that associate with active transcription sites and participate in post-transcriptional mRNA processing. During mitosis, nuclear speckles dissolve following phosphorylation of their protein components. Here, we identify the phosphatases responsible for counteracting kinase-mediated dissolution. We find that overexpression of PP1 phosphatases increases speckle cohesion and leads to retention of polyadenylated RNA within speckles and the nucleus. To characterize the association of specific RNAs with nuclear speckles depending on speckle cohesion, we used proximity-dependent biotin labelling of RNAs in nuclear speckles. We find that many transcripts are preferentially enriched within nuclear speckles compared to the nucleoplasm, particularly chromatin- and nucleus-associated transcripts. However, the RNA composition of nuclear speckles changes minimally with increased cohesion. This suggests that mRNAs are proportionally retained in nuclear speckles in response to dephosphorylation of speckle components. We then explored whether nuclear speckle cohesion changes in response to conditions associated with perturbation of CLK1 or PP1 activity. We found that cellular responses to heat shock, oxidative stress, and hypoxia include changes to the cohesion of nuclear speckles and mRNA retention. Based on our data and comparison to the stress granule transcriptome, we propose a model wherein nuclear transcripts tend to be sequestered within nuclear speckles during stress, while cytoplasmic transcripts are sequestered within stress granules.

Session 4: Development

Regulation of RNA granule assembly and function
Geraldine Seydoux
Johns Hopkins University

I will discuss how P granules, RNA granules in the C. elegans germline, are assembled and function to specify germ cell fate.

The spark of life. Initiating transcription in embryos.
Nadine Vastenhouw
University of Lausanne

The Vastenhouw-lab studies how the transcriptional machinery and chromatin template are brought together in time and space to robustly regulate transcription during development. After fertilization, animals go through cleavage divisions that transform the one-cell egg into a multicellular embryo. During this phase, the genome is inactive, and embryos rely on the products their mothers provided them. During the maternal to zygotic transition, developmental control is handed from maternally provided gene products to those synthesized from the zygotic genome. The onset of transcription is an excellent system to determine how all the different variables that influence the decision to transcribe a gene or not come together to generate complex transcriptional programs. Here, Nadine Vastenhouw will discuss the role of nuclear organization in transcription regulation.

Non-coding RNAs drive cytoplasmic compartmentalization and neuronal function
Valérie Hilgers
Max Planck Institute of Immunobiology and Epigenetics

Neurons are highly polarized cells of complex and dynamic architecture. Projecting axons and dendrites and establishing numerous synapses, neurons constantly adapt to their environment and to external stimuli. Hence, in neurons, it is particularly critical to coordinate gene expression in a robust but dynamic manner. To achieve this level of complexity, neurons employ mechanisms that increase RNA regulatory potential: alternative splicing, alternative polyadenylation, and non-coding RNA expression. In my talk, I will present our published and ongoing work on the role of non-coding RNAs in the compartmentalization of the neuronal cytoplasm, and how this contributes to synaptic function. RNA-binding proteins and messenger RNAs assemble into ribonucleoprotein (RNP) granules that regulate mRNA trafficking, local translation, and turnover. The dysregulation of RNA-protein condensation disturbs synaptic plasticity and neuron survival, and has been widely associated with human neurological disease. In recently published work, we report a novel mechanism of granule formation in neurons: through an architectural RNA. We show in Drosophila that a previously uncharacterized long non-coding RNA, mimi, is required to scaffold large RNP condensates in the adult nervous system. Granule loss in mimi mutant flies impairs nervous system maturity, neuropeptide-mediated signaling and causes phenotypes of neurodegeneration. I will also show some of our unpublished work, in which we demonstrate the differential localization of messenger RNAs with alternative 3' untranslated regions (3' UTRs) in distinct subcellular compartments of Drosophila neurons. Our results reveal UTRdependent mechanisms of functional diversification of the neuronal proteome, strategies that prevent disarray in an environment of extreme RNA diversity, and help translating complexity into cellular plasticity and versatility.

Growth control by an m6A/YTHDF axis in plants
Laura Arribas-Hernandez
University of Copenhagen

Post-transcriptional regulation by modifications of internal nucleotides in mRNAs is of paramount importance in biology. These chemical marks can modulate the affinity between the modified mRNAs and specialised mRNA-binding proteins that act as 'readers' of the modification. The best characterised N6-methyladenosine (m6A) readers are YT521-B homology (YTH) domain proteins. In mammals, cytoplasmic m6A-mRNA regulation by YTH domain proteins of the YTHDF clade regulates pluripotency factors in a process that is indispensable for embryo development, and is associated with neurodevelopmental diseases and cancer. The plant YTHDF homologs, called ECTs in arabidopsis, promote growth by boosting the proliferation of primed stem cells in the primordia of lateral organs such as leaves, flowers and roots. However, the specific genes whose regulation by this pathway is responsible for the stimulation of the growth program remain ill-defined, a difficult problem given that over 6000 genes are targets of the m6A-ECT2/3/4 module. For that reason, genetic ablation of ECT2/3/4 or m6A-deposition factors results in a complex syndrome that so far has precluded a deeper understanding of this process. I will present novel results from isolated plant stem cells that reveal, with striking simplicity, how the growth boost is achieved in plants by this fascinating regulatory system.

PhD workshop pt.2

Unistrand piRNA clusters are an evolutionary conserved mechanism to suppress endogenous retroviruses across the Drosophila genus
Jasper van Lopik
University of Cambridge

The PIWI-interacting RNA (piRNA) pathway plays a critical role in preventing endogenous genomic parasites, transposable elements (TEs), from damaging the genetic material of animal gonadal cells. Dedicated regions in the genome, called piRNA clusters, define each species' piRNA repertoire and thereby its ability to recognize and silence distinct transposon families. The Drosophila ovary is constituted of germ cells and somatic cells. In the somatic compartment of the Drosophila melanogaster ovary, the flamenco (flam) unistrand cluster is the primary source of piRNAs. Flam predominantly regulates Gypsy family TEs, capable of forming virus-like particles that enable them to infect the neighbouring germ cells. Disruption of the flam locus or failure to process flam precursor transcripts into piRNAs can result in sterility. Despite flam's indispensable role in TE regulation in Drosophila melanogaster, it remains unknown whether this silencing mechanism is employed widely across Drosophilidae. Here, we will show that using both synteny-based analyses and de novo TE annotation, we were able to identify candidate loci sharing both their organisation and TE targeting repertoire with flam in widely divergent Drosophila species groups. Small RNA-sequencing validated these loci as bona fide unistrand piRNA clusters and revealed their predominant expression in somatic cells of the ovary, likely to counter TE mobilisation in this tissue. We have found compelling evidence for co-evolution between virus-like Gypsy family transposons and a host defence mechanism in the form of soma-expressed unistrand piRNA clusters. We will also show that these unistrand piRNA clusters all evolved to specifically repress Gypsy family TEs with the capacity to transmit from cell-to-cell. In conclusion, our discoveries will allow for the first time to address several long-standing hypotheses regarding piRNA cluster emergence, transcriptional regulation, and how their transcripts are fated for piRNA biogenesis.

3'UTR mRNP repositioning triggers selective mRNA decay to drive rapid embryonic morphogenesis
Klara Kuret
National Institute of Chemistry

Prior to gastrulation, the mammalian embryo undergoes a rapid morphological transformation, in which naïve precursor cells assemble into a rosette structure. This cell-fate transition requires a pivot from WNT to MEK/ERK signalling, which leads to depletion of key transcription factors that maintain the naïve pluripotency expression programme. Our research identifies LIN28A as a key effector that enables cells to respond to this shift in signalling dynamics through MEK-induced phosphorylation of its intrinsically disordered region (IDR). Comparative analysis of LIN28A iCLIP data before and after activation of MEK/ERK signalling revealed that phosphorylation of LIN28A-IDR significantly alters its RNA-binding preferences, promoting binding to long U-rich stretches and simultaneously decreasing binding to canonical GGAG motifs recognized by LIN28A ZnF domain. This shift in RNA-binding specificity correlates with a dramatic repositioning of LIN28A to the AUU-rich termini of 3'UTRs, which are also occupied by poly(A)-binding complex. We identify the density of these terminal AUU-rich motifs as a key feature that selects transcripts for targeted mRNA decay. Interestingly, our findings suggest that the relocation of LIN28A to these motifs coincides with increased interaction of poly(A)-binding complex with these sites. Overall, our work highlights the potency of signal-dependent RNP reassembly to coordinate cell fate by mediating selective mRNA decay. Additionally, our research offers mechanistic insights into the interplay between LIN28A and known effectors of mRNA stability in early embryonic development.

Activation of antiviral RNAi in mammals
Marcos Iuri Roos Kulmann
Institute of Molecular Genetics

RNA interference (RNAi) mediates sequence-specific degradation of cognate RNAs. For that, long double-stranded RNAs (dsRNAs) are processed by Dicer into small interfering RNAs (siRNAs) that guide cleavage of perfect complementary targets. Besides regulating gene expression and suppressing mobile elements, RNAi is an ancestral antiviral response in plants and invertebrates. However, the relevance of antiviral RNAi in mammals remains controversial because: i) mammals have evolved an Interferon-based innate immunity; and ii) mammalian Dicer is structurally adapted to process microRNA precursors, but not long dsRNAs. Here, we show that enhancing Dicer activity is sufficient for activation of antiviral RNAi in mammals. We found that expression of DicerΔHEL1, known to facilitate dsRNA processing, increases viral siRNA production and confers antiviral protection in cells. For testing a similar principle in vivo, we developed a mouse model with transgenic expression of DicerΔHEL1 from the Rosa26 locus. Remarkably, DicerΔHEL1 led to activation of antiviral RNAi in vivo, protecting animals during Lymphocytic Choriomeningitis Virus (LCMV) infection. We present a proof of concept for functional in vivo antiviral RNAi in adult mammals and show the key limiting factor is having sufficient Dicer activity. The activation of antiviral RNAi provides an additional layer of immunity in mammals.

True or false, heads or tails, SNP or error, mobile or not; And the implication for determining the mobility of mRNA
Franziska Hoerbst
John Innes Centre

"Studying single nucleotide polymorphisms (SNPs) has become extremely useful in many areas of biology, from GWAS to molecular evolution to long-distance RNA transportation in vascular plants. All these analyses, however, depend on distinguishing potential sequencing errors from true SNPs. We constructed a statistics pipeline for differentiating sequencing errors from actual SNPs tailored to our main use case: the identification of graft-mobile mRNA. Hundreds, maybe thousands of RNAs are transported over long distances through plants and between parasitic plants and their hosts. One hypothesis for their transport is that they are acting as signalling molecules conveying messages from on part of the plant to another. To test this involvement, however, we need to be able to detect and trace them. In numerous experiments, different ecotypes of Arabidopsis thaliana are grafted together, and mRNAs travelling around between the two ecotypes across the graft-junction can be detected by their ecotype specific SNPs. RNA-seq performed on samples taken from all over the grafted plant give us information about where specific alleles are populated. However, separating the sequencing errors from the SNPs has previously been an area of risk for quality, as population numbers of the mobile mRNAs can be very low and RNA sequencing errors can't be fully eradicated. In our new statistical pipeline for the RNA-Seq data, we input position specific error probabilities and infer expected sequencing error distributions for all of them. By calculating a Bayes factor for each SNP, we compare the evidence for the expected sequencing errors to the sequencing data in which we are looking for specific SNPs. In that way, we can identify the positions where solely sequencing errors cannot explain the variability we see – we identify the true SNPs. We tested this method extensively on simulated data and found really strong sensitivity and specificity in the performance of our exact Bayesian inference approach. Furthermore, we used the methods to re-analyse published mRNA-mobility data and identified a list of high confidence mobile transcripts. These mobile transcripts and their transport are now ready to be studied intensively to test their potential involvement in signalling processes."

PhD workshop pt.3

Guide-RNA sequence determinants of Argonaute2 slicing kinetics and conformational dynamics
Peter Wang
Whitehead Institute

Human Argonaute2 (AGO2) associates with ~22-nt guide RNAs, such as miRNAs or synthetic siRNAs. When the associated guide RNA base-pairs extensively to a target RNA, AGO2 can slice the target, leading to its degradation. This slicing activity is at the heart of RNAi-based research reagents and therapeutics, and is occasionally directed by some human miRNAs. Slicing is also the primary mode of action for many AGO2 orthologs, including those that associate with miRNAs and endogenous siRNAs in plants, or endogenous siRNAs in fungi, insects, nematodes, and mice. Recent work defined the slicing kinetics and base-pairing requirements of AGO2 for two guide sequences. However, how the kinetics of slicing might vary for other guide sequences has been unclear. We measured the slicing kinetics of AGO2 loaded with 29 different natural guide sequences or their derivatives. Among these sequences, we found a >250-fold range in the single-turnover kcat values when slicing perfectly complementary targets. Analyses of these results identified four sequence determinants that each conferred a 1.5- to 9-fold difference in kcat, and one that conferred a 600-fold difference in the tolerance for mismatches to the 3′ nucleotides of the guide. These determinants correlated with the strength of knockdown in cells, and some coincided with sequence features previously found in effective siRNAs, thereby providing a mechanistic basis for siRNA design guidelines used for the past two decades. When mapped to AGO2 structure models, these determinants also provided insight into the conformational requirements of slicing. To understand the effect of sequence determinants on conformational dynamics, we probed these dynamics using hydroxyl-radical footprinting and found that the kinetics of slicing perfectly complementary targets is limited by the kinetics, not the thermodynamics, of associated conformational changes. Together, these results delineate the biochemical, conformational, and functional basis for sequence determinants of AGO2 activity and RNAi efficacy.

Epitranscriptomic fingerprinting: tissue and cell type deconvolution from rRNA modification patterns
Ivan Milenkovic
Centre for Genomic Regulation

RNA modifications are functional groups that decorate RNA molecules, changing their chemical and biological properties. Ribosomal RNA (rRNA) are one of the most heavily modified RNA species, with over 220 modified nucleotides identified in human 18S and 28S rRNA. However, whether rRNA modifications vary across cell types, tissues, developmental stages, or upon disease states, is largely unknown. In this regard, individual works have started to point towards rRNA modifications being dynamically regulated under specific conditions, but a comprehensive survey of how the rRNA modification landscape is regulated in a time, cell type and stimuli-dependent manner, is still largely absent. Here, we employ direct RNA nanopore sequencing to generate comprehensive maps of rRNA modifications, their abundance and dynamics across multiple tissues, developmental stages and cell types. We identify multiple rRNA sites that are dynamically modified across tissues and/or developmental stages, suggesting that rRNA modification patterns have additional levels of complexity. Furthermore, we find that the adult brain has the most distinct rRNA modification patterns, and identify several previously unannotated brain-specific rRNA modifications. Moreover, we identify a single ribose methylation site, 18S:Um355, whose methylation status is inversely correlated with the proliferation potential of the cell of origin. We then demonstrate that these dynamic rRNA modification patterns are inherent to tissues and cell type of origin, and thus can be used for tissue and cell type identification, which we hereby term 'epitranscriptomic fingerprinting'. As a proof of principle, we show that a simple linear discriminant analysis model successfully classifies samples into tissue type and developmental stage based on rRNA modification patterns, with an overall accuracy of 81%. Altogether, our results reveal a previously unexplored avenue for exploiting rRNA modifications as a source of information for tissue and cell type identification and deconvolution."

Everything Everywhere All at once? Understanding transposon silencing in the minute flowering plant Wolffia
Daniel Buendia Avila
Gregor Mendel Institute for Molecular Plant Biology

Transposable elements (TEs) or transposons, are mobile DNA sequences capable of replicate within host genomes. TEs are silenced by numerous epigenetic mechanisms, being the deposition of DNA methylation one of the most relevant in plants. In angiosperms, the RNA-directed DNA methylation (RdDM) pathway plays an important role in depositing de novo DNA methylation on TEs through the production of 24nt-siRNAs. However, the duckweed family (Lemnaceae) appears to be an exception. Duckweeds represent the smallest and fastest growing flowering plants known to date, thanks to rapid asexual clonal propagation. During clonal propagation, several of its members show no expression of RdDM components, low levels of 24nt-siRNAs, and low DNA methylation associated with RdDM activity although TEs remain silenced. To fully understand TE silencing mechanisms in duckweeds, we have expanded our investigation to one of the most recent lineages of the family: Wolffia brasiliensis. Wolffia brasiliensis has a relatively high TE content and show recent bursts of transposition compared to the other duckweeds investigated. Like other duckweeds, Wolffia exhibits no RdDM expression and associated DNA methylation signatures. However, in contrast to the other species, 22nt-siRNAs can be found arising from TE loci alongside 24nt-siRNA in several instances. In plants, 22nt siRNAs are associated with Post-Transcriptional Gene Silencing (PTGS) while 24nt are involved in RdDM mediated Transcriptional Gene Silencing (TGS), two pathways considered as mutually exclusive. Whether both siRNA classes share a common source of siRNA precursors, or the different silencing pathways in Wolffia follow a tissue specific expression pattern, remains unknown so far. These unique features of duckweeds, and Wolffia in particular, offer an exceptional opportunity to investigate TE silencing and their interplay with their hosts in non-model plant organisms.

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Session 5: Small RNAs

New functions and regulators of noncoding RNAs in mammals
Joshua Mendell
UT Southwestern Medical Center

Long noncoding RNAs (lncRNAs) have been proposed to perform a diverse array of cellular functions, including regulation of transcription in cis or in trans, structural organization of sub-cellular domains, and direct regulation of other RNAs or proteins. Nevertheless, the subset of detectable lncRNAs that actually perform RNA-mediated functions, and the molecular nature of those potential functions, remains unclear. Our laboratory has been focused on identifying and functionally characterizing lncRNAs that leave the site of transcription and perform robust RNA-mediated functions in trans in mammalian cells. Although it is likely that a minority of lncRNAs fall into this class, identification of those that do has revealed unexpected RNA-mediated functions that impact normal physiology and disease. In this talk, I will present our latest data resulting from our efforts to uncover new regulators and functions of trans-acting lncRNAs in mammalian cells.

MUT-7: a conserved exoribonuclease involved in sRNA biogenesis
Virginia Busetto
Max Perutz Labs / University of Vienna

The MUT-7 family of 3'-5' exoribonucleases is conserved through the animal kingdom. In C. elegans, MUT-7 is part of the Mutator complex, which localizes to perinuclear germ granules (Mutator foci), whose formation depends on the Mutator factor MUT-16. Moreover, MUT-16 acts as a hub recruiting the other components of the complex, which work together in a process known as sRNA amplification. sRNA amplification produces 22G RNAs that, together with Ago proteins, silence transposable elements (TEs). Mutations in MUT-7 or any other Mutator component compromise 22G RNA levels, resulting in TE de-repression. While the function of the fly MUT-7 ortholog Nibbler in trimming the 3' end of sRNAs in the Nuage is established, the precise role of CeMUT-7 in the sRNA amplification process and the function of MUT-7 orthologs in vertebrates remain unknown. Interestingly, Nibbler lacks the highly conserved C-terminal domain (CTD) that is present in all other MUT-7 orthologs and whose function is unknown. Using purified proteins, we show that the CeMUT-7 CTD binds to MUT-8, a nematode-specific factor proposed to recruit MUT-7 to MUT-16 by in vivo studies. We demonstrate that MUT-8 uses its folded CTD to bind MUT-7 CTD and its unstructured N-terminal domain to directly contact MUT-16 via condensate formation. Thus, MUT-8 acts as an adapter bridging MUT-7 to MUT-16/Mutator foci. Since Mutator complexes are not present in animals besides worms, this suggests that the MUT-7 CTD should have a different purpose than simply facilitating MUT-8 binding. Our RNA-binding experiments suggest that the MUT-7 CTD contributes to RNA binding in both CeMUT-7 and its vertebrate homolog EXD-3. Overall, this study identifies MUT-8 as the first direct partner of the scaffold MUT-16 and assigns both an RNA-binding and a recruiting function to MUT-7 CTD.

Transposable element regulation and DNA damage response during germline development
Felipe Teixeira
University of Cambridge

Transposable elements (TEs) harbour a selfish drive to increase in copy number in host genomes. Due to its role in inheritance, the germline is the platform TEs use to maximize their influence over genetic information passed between generations. The Drosophila P-element DNA transposon is one of the best-studied eukaryotic TEs, providing a unique system for dissecting transposon regulation and the consequences of TE invasions in animals. One of the critical aspects of P-element biology is how fast and extensive these elements were shown to spread through wild populations, highlighting their capacity to invade but also to accommodate the host genome. In this system, germline protection is mediated by epigenetically inherited small RNAs (sRNAs) cognate to the P-element, which are strictly maternally provided. Indeed, without such protective small RNAs, P-elements become active and induce several germline-specific phenotypes collectively known as P-M hybrid dysgenesis - a classic example of how transposon activity can affect germline development and lead to sterility. We used this system to understand the molecular mechanisms by which germ cells use small RNA-based pathways to protect their genomes against transposable elements, as well as how germ cells sense and respond to DNA damage once transposons evade the protective mechanisms.

Eclipse Bioinnovations: Accelerating the RNA genomics discoveries
Katarzyna Kuduk
Eclipse Bioinnovations

Novel RNA genomics technologies go beyond traditional RNA-Seq and illuminate structural and regulatory aspects of RNA as well as the RNA interactome. Our presentation will introduce Eclipse Bio's data-driven, NGS-based technologies that interrogate RNA structure, RNA interactions with RNA binding proteins, RNA modifications such as m6A, ribosome occupancy and microRNA-mRNA interactions.

RNA modifications in control of mammalian gene expression
Ramesh Pillai
University of Geneva

N6-methyladenosine (m6A) is an essential internal RNA modification that is critical for gene expression control in most organisms. They are catalyzed by RNA methyltransferases 'writers' on specific targets, while protein 'readers' with a YTH domain recognize the m6A marks to mediate molecular functions like RNA splicing, mRNA decay and translation control. The functional relevance of these marks is demonstrated by the ability of RNA demethylase 'erasers' to remove this mark, pointing to potential reversibility and regulation. Here we describe the crystal structure of the writer human METTL16 to reveal a classical methyltransferase domain but with an extra N-terminal module that is essential for catalysis. Together, they form a deep-cut groove lined by highly conserved positively charged residues that are essential for RNA binding and methylation activity. When given a random pool of RNAs, METTL16 selects structured RNAs for m6A methylation. We demonstrate that mouse Mettl16 is essential for early embryonic development, and acts via regulation of the SAM synthetase Mat2a mRNA. Our results highlight the pivotal role of an m6A RNA methyltransferase in facilitating early developmental decisions via regulation of SAM availability.

Session 6: Translation control

RNA structure, a hidden regulator in living cells
Yiliang DING
John Innes Centre

RNA structure plays an important role in the post-transcriptional regulations of gene expression. The Ding group has been studying the functional roles of RNA structure in diverse biological processes such as mRNA processing (splicing and polyadenylation), translation and RNA degradation. The Ding group has also developed new methods to reveal the existence of tertiary RNA G-quadruplex structures in eukaryotes and uncovered that RNA G-quadruplex structure serves as a molecular marker to facilitate plant adaptation to the cold during evolution. Recently, the Ding group has developed the single-molecule RNA structure profiling method and revealed the functional importance of RNA structure in long noncoding RNAs.

Immediate targeting of host ribosomes by jumbo phage encoded proteins
Milan Gerovac
Institut für Molekulare Infektionsbiologie

Bacteriophages must seize control of the host gene expression machinery to promote their own protein synthesis. Since the bacterial hosts are armed with numerous anti-phage defence systems, it is essential that mechanisms of host take-over act immediately upon infection. Although individual proteins that modulate components of the bacterial gene expression apparatus have been described in several different phages, systematic approaches which capture the phage's arsenal for immediate targeting of host transcription and translation processes have been lacking. In particular, there are no known phage factors that associate directly with host ribosomes to modulate protein synthesis. Here, we take an integrative high-throughput approach to uncover numerous new proteins encoded by the jumbo phage ΦKZ that target the gene expression machinery of the Gram-negative human pathogen Pseudomonas aeruginosa immediately upon infection. By integrating biochemical and structural analyses, we identify a conserved phage factor that associates with the large ribosomal subunit by binding the 5S ribosomal RNA. This highly abundant factor is amongst the earliest ΦKZ proteins expressed after infection and stays bound to ribosomes during the entire translation cycle. Our study provides a general strategy to decipher molecular components of phage-mediated host take-over and argues that phage genomes represent a large discovery space for proteins that modulate the host gene expression machinery.

The ribosome: when RNA meets protein to sense small molecules
Axel INNIS
Institut Européen de Chimie et Biologie (IECB)

In order to detect and respond to varying concentrations of metabolites in their environment, cells rely on a multitude of proteins and structured RNAs, such as transcription factors or riboswitches. In some cases, metabolite sensing is achieved by a combination of protein and RNA, in the form of ribosomes engaged in the synthesis of specific amino acid sequences. Such ribosome-arresting peptides (or arrest peptides for short) cause ribosomes that translate them to stall on the mRNA in a metabolite-dependent manner, which in turn regulates the expression of downstream genes through co-transcriptional or translational mechanisms. Although metabolite-dependent arrest peptides have been known for decades, the mechanisms used by translating ribosomes to sense different metabolites have only recently become apparent with the advent of high-resolution cryo-EM. Furthermore, the extent to which arrest peptides regulate gene expression in response to various metabolites is still unknown. Here, I will present efforts by our group to reveal the basic strategies by which arrest peptides recognize various metabolites, and I will describe ongoing efforts to determine the extent and diversity of metabolite-sensing arrest peptides in nature.

Poster Abstracts

Please note that although all posters will be exhibited at the same time, the presentations will take place on different days. Please refer to the lists below for the day of your presentation.

SESSION 1 – Thursday May 04th

NR	Name	Poster title
1	Jakub Dolata	Co-transcriptional miRNA biogenesis in plants
3	Rohit Nalawade	A conserved miRNA turnover mechanism regulates the oncogenic potential of specific cancer types
5	Roman Renger	Visualizing and manipulating protein-RNA interactions on the single molecule level
7	Laura Lorenzo-Orts	Storage and repression of maternal mRNAs in the oocyte and early embryo
9	Kristin Röhrborn	The contribution of salivary extra-cellular vesicles to taste-cell transcriptomics
11	Rupert Faraway	RNA multivalency drives homeostasis of low complexity charged domains
13	Júlia Portell Montserrat	In vitro reconstitution of actively silencing PIWI complexes
15	Ashwin Narain	SPT6 directly regulates RNAPII transcription elongation and termination
17	Tibor Csorba	NODULIN HOMEOBOX is required for heterochromatin homeostasis in Arabidopsis
19	Annamaria Sgromo	Systematic profiling and kinetic modeling of uridylation reveals molecular principles of RNA quality control in Drosophila
21	Marek Sebesta	Trimeric SOSS1 complex promotes transcription at double-strand breaks
23	Rodolphe Dombey	Transposon silencing in Spirodela polyrhiza, for whom size matters.
25	Denis Mustafov	Downregulation of miR-21 and CORO1C in glioblastoma cells upon treatment with a new carbonyl compound
27	Valentin Mitterer	ATPase-dependent maturation of 60S pre-ribosomes
29	Sarah Salah	STUDY OF SERUM PIRNA-54265 AS A NON-INVASIVE BIOMARKER FOR COLORECTAL CANCER IN EGYPTIAN PATIENTS
31	Jutta Hafner	Upa1 and Upa2 are part of an unconventional snoRNP
33	Zdenka Hasanova	Human senataxin is a bona fide R-loop resolving enzyme and transcription termination factor
35	Jana Orlíčková	Deep sequencing of circulating microRNAs in pediatric patients with juvenile and adolescent idiopathic scoliosis
37	Tamás Orbán	The potential role of exosomal small-RNAs in the early prediction of Preeclampsia combined with Intrauterine Growth Restriction
39	Vienna Huso	In vitro reconstitution of mixed lineage kinase zak activation and regulation
41	Lenka Stixova	PARP-dependent and NAT10-independent acetylation of N4-cytidine in RNA appears in UV-damaged chromatin
43	Josef Roehsner	Deciphering the function and regulation of dormant ribosomes
45	Pranjali Bhandare	EU incorporation-based screening reveals several novel transcription regulators
47	PRADEEP PANT	Design and Characterization of Flexible Nucleic Acids for Disrupting Viral Counter-Defense Machinery
49	Jana Brunner	LIN-28 as a conserved factor controlling the onset of animal puberty
51	Maria Salinas	Comparison of miRNA expression in PGC cell cultures before and after freezing in different freezing media
53	Malgorzata Rak	Pro-survival role of LINC00116-encoded peptide Mitoregulin in Hodgkin lymphoma
55	Pavla Musilova	Adar null mutant mice lacking the Adar1 RNA editing enzyme: effects of cell death mutations on survival and innate immune defect
57	Igor Kaczmarczyk	Structural analyses of the human QTRT1/2 complex
59	Magdalena Fickl	6-Thioguanosine monophosphate prodrugs with the potential to overcome thiopurine resistance
61	Maria Zlobina	High-throughput analysis of small drug-like molecules interacting with mRNA 3'UTRs
63	Mohd Isar	RNA as a therapeutic target
65	Jakob Püschel	Understanding the differences in ribosome binding capabilities of two dormancy factors Dap and Dap1b
67	Daniel Gebert	Transposable elements and the forces driving genome evolution
69	Christian Ramirez Amarilla	SNOMATCHER: IDENTIFICATION OF CANDIDATE GUIDE SNORNAS FOR NEWLY-DISCOVERED 2'-O-METHYLATIONS

SESSION 2 – Friday May 05th

NR	Name	Poster title
2	Ernesto Aparicio-Puerta	A comprehensive atlas of human and mouse small non-coding RNAs reveals conserved tissue-specific expression patterns
4	Claudia dos Santos Martinho	Deciphering the molecular mechanisms underlying virus-brown algae interactions
6	Dominik Handler	Drosophila somatic piRNA precursor selection – exploiting a weak spot of somatic retrotransposons
8	Farja Isabel Ayala Munoz	Mechanistic insights on how the human mRNA export factor recognizes mRNPs
10	Devansh Raj Sharma	Conservation of miR-100 and the let-7-complex locus in the nematodes Pristionchus pacificus and Caenorhabditis elegans
12	Rahul Mehta	Structural and biophysical characterization of non-coding RNAs
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POSTER ABSTRACTS

POSTER NUMBER: 1

Co-transcriptional miRNA biogenesis in plants

Jakub Dolata
Adam Mickiewicz University

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

POSTER NUMBER: 2

A comprehensive atlas of human and mouse small non-coding RNAs reveals conserved tissue-specific expression patterns

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Small non-coding RNAs (sncRNAs) are key regulators of gene expression and play important roles in various biological processes and diseases. However, the evolution of sncRNA expression patterns across mammals is still not well understood. Here we first present a comprehensive study, aiming to build an atlas of the mammalian sncRNA transcriptome from publicly available miRNA-seq data. We have already compiled data from 100,000 publicly available samples. As a first approach, we analyzed 407 billion sequencing reads from 43,000 samples covering over 50 human and mouse tissues. We compared expression patterns using an uniform system for the annotation and nomenclature of miRNA genes based on MiRGeneDB, a curated database of miRNA gene families across vertebrates. We also identified isomiRs (sequence and/or length variants of miRNAs) annotated on isomiRdb. Finally, we performed a general comparative analysis on the distribution of other RNA types and revealed cross-species tissue-specific expression patterns. Our study lays the foundation for a comprehensive and consistent atlas of the mammalian sncRNA transcriptome, an important resource to reveal new insights into the annotation and evolution of miRNA genes and isoforms.

POSTER NUMBER: 3

A conserved miRNA turnover mechanism regulates the oncogenic potential of specific cancer types

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MicroRNAs are small non-coding RNAs that are extensively involved in regulating diverse developmental and physiological processes. A consistent link between the dysregulation of miRNAs and various diseases highlights the importance of robust regulation of miRNA activity. Biogenesis of mature miRNAs and their regulation have been comprehensively studied. However, molecular pathways leading to the decay of these small RNAs remain unexplored. We demonstrate that ribonuclease XRN2 is directly involved in the turnover of mature miRNAs in multiple human cancer cells. We explore the capability of human XRN2 as a 'miRNase' and elucidate the molecular mechanism underlying the oncogenic potential of XRN2 in relation to its 'miRNase' activity. Depletion of XRN2 leads to the accumulation of AGO-bound miRNAs that, in turn, downregulates their cognate targets, indicating that XRN2-mediated turnover of mature miRNAs occurs downstream of the process of disruption of the AGO2-miRNA complex. Substantial increase in the levels of mature miRNAs upon XRN2 knockdown in different cancer cells, with a concomitant effect on their crucial target-oncogenes, is endorsed by a prominent impact on the tumorigenicity of the cancer cells. Experiments in athymic mice illustrate a drastic reduction in tumor growth upon XRN2 depletion, which was prominent in the case of glioblastoma cells. Our biochemical assays indicate that XRN2-mediated degradation of mature miRNAs happens upon release of miRNAs from AGO by an unknown protein/s, and these two steps are kinetically linked. Our results suggest that the release of miRNAs from the grasp AGO and its subsequent degradation by XRN2 occurs majorly in the nuclear compartment of the cell. Inside the nucleus, XRN2 associates with a nucleolar protein, NOP58, which functions as an RNA binding factor, thereby ensuring XRN2's nuclease activity to act specifically on miRNAs. We also demonstrate that our cell line-based observations significantly correlate with the clinical data of cancer patients from TCGA datasets. These analyses also explain previous observations of elevated XRN2 mRNA expression being associated with worse survival in cancer patients. Collectively, our study reveals that human miRNAs are regulated by a two-step turnover pathway, wherein XRN2 plays the role of a 'miRNase' in various tissues.

POSTER NUMBER: 4

Deciphering the molecular mechanisms underlying virus-brown algae interactions

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In marine environments viral particles are ubiquitous and accumulate in the range of 107-109 per mL of seawater. Phaeoviruses are dsDNA virus belonging to the super group of Nucleocytoplasmic large DNA viruses (NCLDVs) with very diverse shapes and encoding hundreds of proteins required to sustain complex virus-host interactions. Phaeovirus infections have been described in kelps and

multiple other brown algae families. In particular, the interaction between the filamentous brown alga Ectocarpus and the Ectocarpus siliculosus Virus-1 (EsV-1) and has been a model for DNA viral infection in brown algae. Ectocarpus zooids are susceptible to EsV-1 de novo infection but the alga vegetative tissues remain disease-free until the development of the algal reproductive structures. At the onset of fertility, viral replication is triggered exclusively in a subset of sporangia or gametangia, which consequently present disease symptoms. The virus is transmitted horizontally to the next generation by the highly synchronous release of viruses and gametes to the environment. Esv-1 may also be transmitted vertically to the progeny via genomic insertions and mendelian segregation of the inserted viral element.
Although the EsV-1 infection cycle has been well described in the past, the exact molecular mechanism underlying virus silencing in vegetative tissues, activation and synchronization with the algae life-cycle remain unknown to date. We will describe how we are using, genomics and transcriptomics to reveal the molecular mechanisms underlying viral silencing in vegetative tissue and the further release of silencing specifically in reproductive structures. Our results are consistent with EsV-1-derived EVEs RNA playing a key role in algae-virus interactions. In addition, we have identified candidate genes likely to participate in anti-viral defence.

POSTER NUMBER: 5

Visualizing and manipulating protein-RNA interactions on the single molecule level
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LUMICKS B.V.

Biological processes performed by proteins interacting with RNA are key to cell health and viability. A detailed understanding of the relation between structure, conformational states and function of these biomolecules, as well as the functional role in membrane-less intracellular compartmentalization, is essential for this journey towards a more complete picture of the diverse roles of protein-RNA interactions in health and disease.
Direct, real-time observations of individual proteins interacting with RNA are required to validate and complete the current biological models. Single-molecule technologies offer an exciting opportunity to meet these challenges and to study RNA-dependent protein function and activity in real-time.
Here, we present our efforts for further enabling discoveries in the field of protein-RNA interactions using the correlative combination of optical tweezers with fluorescence microscopy and microfluidics. We present several examples in which our technologies enhanced the understanding of RNA-based DNA editing tools, translational regulation and RNA-protein condensates. Furthermore, we demonstrate that advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new avenues in the field of protein-RNA interactions.

POSTER NUMBER: 6

Drosophila somatic piRNA precursor selection – exploiting a weak spot of somatic retrotransposons
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The PIWI-interacting RNA (piRNA) pathway suppresses the activity of transposable elements in all animal lineages. The precise loading of PIWI-clade Argonaute proteins with transposon complementary piRNAs is critical for its function. We are investigating the process of piRNA precursor selection in Drosophila ovarian somatic cells. Here, piRNA synthesis initiates de-novo during development and a single PIWI family protein, Piwi, is loaded with 23-31 nt piRNAs processed predominantly from a several hundred kilobase transcript originating from the flamenco locus. While previous work has implicated the RNA helicase Yb in piRNA precursor selection, the molecular features within the precursor RNA recognized by Yb are not known.
We used a cell line derived from follicle stem cells (OSCs) recapitulating the somatic ovarian piRNA pathway. To define piRNA source loci, we sequenced and assembled the genome of the OSC cell line. Based on this, ~ 70% of all piRNAs are derived from piRNA clusters and 25 % from genic mRNAs. In wild-type cells, no strict correlation exists between piRNA levels and cellular RNA abundance. Upon depletion of Yb, piRNA levels and precursor RNA abundance are more correlated, indicating the existence of specificity features read out by Yb. Computationally we identified that uridine-rich transcripts are preferentially processed into piRNAs. This Uridine dependency was confirmed in piRNA biogenesis reporter experiments showing stimulation of piRNA biogenesis within transcripts if the local Uridine-content exceeds 25-30%.
Remarkably, the targets of the somatic piRNA pathway, endogenous retroviruses from the gypsy-clade, have genomes strongly enriched in Adenosines. As flamenco captured gypsy sequence fragments in antisense orientation, the flamenco piRNA precursor is among the most Uridine-rich RNAs in the cell.
Altogether, our work indicates how an evolutionary trait of gypsy-type endogenous retroviruses is used as an Achilles heel for the piRNA pathway to launch a highly efficient and specific silencing response.

POSTER NUMBER: 7

Storage and repression of maternal mRNAs in the oocyte and early embryo
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Maternally inherited mRNAs are crucial for protein synthesis during early development. While some maternal transcripts are translated early on, most are repressed by shortening of the polyadenine tails in the oocyte. While in somatic cells, mRNA deadenylation is linked to decapping and degradation, it is currently unknown how the egg uncouples these processes. Here, we report an essential role of the oocyte-specific paralog of the translational factor eIF4E, eIF4E1B, in repressing and storing maternal mRNAs. Mutation of eIF4E1B in zebrafish impairs female germline development. While eIF4E1B binds to the mRNA cap, it does not interact with the translation

initiation factor eIF4G. Instead, eIF4E1B interacts with eIF4ENIF1/4E-T, which triggers localization of eIF4E1B to P-bodies in the embryo. Given the conservation of eIF4E1B in most vertebrates, understanding its mechanism and in vivo function provides novel insights into fundamental post-transcriptional regulatory principles governing early development.

POSTER NUMBER: 8

Mechanistic insights on how the human mRNA export factor recognizes mRNPs

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Nuclear mRNA export in eukaryotes is highly regulated to ensure, that despite the enormous diversity of synthesized transcripts, only fully processed mRNAs are transported. Following transcription, mRNAs undergo a series of processing events, including splicing, and are marked by proteins, called maturation marks. These marks are deposited on the mRNA during each of processing steps and include the Exon Junction Complex (EJC), which is deposited during splicing upstream of splice junctions. The transcription and export complex (TREX) recognizes the maturing mRNA-protein complexes (mRNPs), facilitates their packaging, and licenses the loading of the mRNA export factor NXF1–NXT1 on the mRNA. This enables the nuclear export of NXF1–NXT1–mRNP complexes through the nuclear pore complex (NPC) for translation in the cytoplasm. Yet, how is NXF1–NXT1 loaded selectively onto fully processed mRNPs, but not immature mRNP precursors? Here, I present evidence for a direct protein-protein interaction between NXF1–NXT1 and the RNA-bound EJC. Biochemical data and protein crosslinking coupled to mass spectrometry show that this protein-protein interaction is compatible with the simultaneous binding of NXF1–NXT1 to RNA and to FG-repeats, which make up the permeability barrier of the NPC. While NXF1–NXT1 shows low affinity for naked RNA, consistent with previous reports, its affinity is increased to the RNA-bound EJC. We speculate that premature association of NXF1–NXT1 with RNA-bound EJCs may be prevented by the chaperoning of the maturing mRNP through TREX (Pacheco-Fiallos, Vorländer, et al., Nature, 2023). These data and additional experiments suggest that NXF1–NXT1 may recognize mRNPs through a combination of sequence non-specific and low affinity RNA-interactions and specific protein-protein interactions with mRNP maturation marks, such as the EJC. This could explain how the mRNA export factor NXF1–NXT1 can be both permissive to the great diversity of mRNAs and simultaneously selective to mature mRNPs.

POSTER NUMBER: 9

The contribution of salivary extra-cellular vesicles to taste-cell transcriptomics

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Objective: Obesity might lead to alterations in salivary extracellular vesicle (EV) composition, with consequences on taste-cell transcriptomics. This project aims to characterize alterations in salivary EV number, size distribution and microRNA content from subjects with obesity and normal weight and correlate them with parameters of the metabolic syndrome. With this, we want to identify and validate EV microRNA-target gene interactions in human taste-cells. Methods: Three methods of EV isolation were tested and validated - first, ultracentrifugation, precipitation using ExoQuick chemistry and size exclusion chromatography (SEC). Quantification and measurement of particle size distribution was done using nanosight technology and western blot analyses. Extraction of total RNA was carried out by miRNeasy Micro Kit. Quantification and quality control were done with Bioanalyzer Small RNA and Qubit™ microRNA Assays. RNA with good integrity was taken forward for small RNA sequencing using NovaSeq6000 platform. Results: The best method to isolate EVs from saliva samples is SEC. This enabled the isolation of the highest concentration and purity of the EVs as well as the best yield of RNA including the microRNA fraction. First small RNA sequencing analyses also confirmed the presence of microRNAs within the isolated EV fraction. Conclusion: Our established protocol allows us to isolate and characterize the EV fraction of a small volume of human saliva with consequent RNA sequencing methods. With this we are able to identify differentially expressed microRNAs in EVs from the study subjects which may offer diagnostic and therapeutic potential.

POSTER NUMBER: 10

Conservation of miR-100 and the let-7-complex locus in the nematodes Pristionchus pacificus and Caenorhabditis elegans

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miR-100 is a highly conserved microRNA, found across different bilaterian species, and possibly even originating from the common ancestor of eumetazoans. As such, its absence in the C. elegans genome is intriguing. We identify miR-100 as one of the most abundant microRNAs in juvenile stages of P. pacificus (Ppc-miR-100). Interestingly, P. pacificus genome shows a partial conservation of the polycistronic let-7-complex, found in multiple species except C. elegans, wherein Ppc-miR-100 is found approximately 200 bps upstream of Ppc-let-7, but does not contain Ppc-lin-4 in the same locus. Knock-out of Ppc-miR-100 results in mild but consistent germline defects, a developmental disturbance that is exacerbated in the Ppc-let-7 mutant. Interestingly, Ppc-lin-4 mutants show near normal overall development. Our study provides deeper insights into the field of small RNA biology and molecular evolution, furthering our understanding of the roles of microRNAs in development and nematode evolution.

POSTER NUMBER: 11

RNA multivalency drives homeostasis of low complexity charged domains

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RNA binding proteins (RBPs) often interact with short, degenerate motifs in RNA. Despite the common occurrence of these motifs, RBPs can still bind to specific subsets of RNA. One identified mechanism by which RBPs achieve specific binding is through cooperative multivalent RNA-protein interactions. Despite this, the multivalency potential of RNA sequences has never been thoroughly characterised. Here, we develop an efficient algorithm for the scoring of generalised RNA multivalency (GeRM) potential. We use GeRM to identify different multivalent RNA sequence classes in the protein coding sequences which host different RBPs. Due to the structure of the genetic code and the stereotypy of the sequence, these regions encode specific subtypes of low complexity domain which are predicted to be disordered and are enriched for different gene ontologies.

Here we study the purine-rich multivalent mRNAs that encode low complexity charged domains, including arginine-rich mixed charge domains (R-MCDs) that drive nuclear speckle localisation of proteins. We find that these mRNAs, as expected, interact with nuclear speckle-localised RBPs. We then study the role of these interactions in speckle localisation and nuclear retention by using a diverse reporter library of transcripts encoding an R-MCD. We show that RNA sequence multivalency drives nuclear retention of transcripts in response to changes in the abundance of nuclear speckle proteins, and that this nuclear retention is driven by mRNA and protein localisation in the nuclear speckle. In summary, we show how nuclear speckle localisation can affect export of mRNAs encoding low complexity charged domains.

POSTER NUMBER: 12

Structural and biophysical characterization of non-coding RNAs

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

POSTER NUMBER: 13

In vitro reconstitution of actively silencing PIWI complexes

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PIWI-family proteins bind piRNAs (a class of small RNAs) and together they silence transposons and other selfish elements in animal gonads. Although the pathway has been extensively characterised at the genetic level, the silencing process is poorly understood at the mechanistic level. In my poster, I will present my efforts to reconstitute two PIWI proteins bound to piRNAs and their complementary targets in vitro: the cytoplasmic Aubergine and the nuclear Piwi. I have established an in vitro cleavage assay with Aubergine and I devised a strategy for reconstituting PIWI-target RNA complexes at large scale. Using these systems, I aim to understand which additional cofactors directly bind these complexes by biochemical and structural means.

POSTER NUMBER: 14

Structural basis of metazoan intron-lariat spliceosome recognition and disassembly

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At the end of pre-mRNA splicing, the intron-lariat spliceosome (ILS) is actively disassembled to regenerate spliceosome components for the next round of splicing. However, despite recent efforts (refs 1–4), the mechanisms of intron-lariat spliceosome recognition and disassembly remain incompletely understood. Here, we determine multiple cryo-electron microscopy structures of human and worm intron-lariat spliceosomes between 2.6 Å and 3.4 Å resolution to address this gap. The structures resolve several essential metazoan splicing factors and complete models of NTC, NTR, and IBC complexes. The metazoan intron-lariat spliceosome is recognized for disassembly through the proteins TFIP11, PAXBP1, and CWF19L2. These interfaces are exposed only after spliced mRNA-protein complexes (mRNPs) have dissociated, and can explain how premature spliceosome disassembly is prevented. The data further reveal a network of interactions that control the targeting spliceosome disassembly helicase, DHX15, and may control its activity. Among these, CWF19L2 and the newly identified CWF19L1 and SYF2 proteins aid to conformationally lock the otherwise mobile spliceosome core and precisely position the disassembly helicase DHX15 to initiate regulated disassembly. Collectively, the visualized disassembly factors extend over 200 Å across the spliceosome, and thereby read out spatially distant molecular surfaces to ensure that spliceosome disassembly occurs at the right time and place.

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4. Zhang, X. et al. An Atomic Structure of the Human Spliceosome. Cell 169, 918-929.e14 (2017).

POSTER NUMBER: 15

SPT6 directly regulates RNAPII transcription elongation and termination

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SPT6 is observed to regulate RNAPII transcription by maintaining chromatin integrity (Kaplan et al., Science 2003). However, it also tightly interacts with elongating RNAPII (Vos et al., Nature 2018b). Why SPT6 tightly interacts with RNAPII and whether it has any direct effect on RNAPII transcription is uncertain in mammals. This study aimed to identify the primary functions of SPT6 by targeted protein degradation using auxin-inducible degron system in cultured human cancer cells and engrafted murine cancer cells. After rapidly depleting SPT6, global changes in RNAPII dynamics were studied in cells and tumours using 4sU-seq, ChIP-Rx, SLAM-seq, RNAPII-IP-Mass Spectrometry and 4TU-TALK-seq. This led to 3 key findings.

First, acute loss of SPT6 led to global decrease in RNAPII processivity and productive transcription. Unlike acute loss, only sustained loss of SPT6 led to cryptic initiation – a phenomenon observed due to chromatin integrity loss. Second, SPT6 loss led to impaired RNAPII termination on all protein coding genes. Quantitative Mass Spectrometry showed that SPT6 might be required for RNAPII interaction with termination factors such as XRN2 and CSTF2. Third, 4TU-TALK-seq demonstrated the RNAPII processivity and termination defects on SPT6 loss were conserved in tumours. Moreover, absence of SPT6 led to decreased splicing efficiency of RNA.

In conclusion, this study shows that SPT6 has dual role in RNAPII transcription. First, it maintains RNAPII processivity, termination and splicing efficiency. Second, SPT6 maintains chromatin structure and only its sustained loss leads to epigenetic chaos causing cryptic initiation.

POSTER NUMBER: 16

Mechanistic Understanding of picd-1 in Transposon Silencing and Genome Maintenance

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Genome integrity is constantly threatened by internal and external corrosive agents including transposable elements (TEs) and repetitive sequences. Hence, it is vital to understand the mechanism(s) of TE transposition and its regulation given the prevalence of TEs and their negative effects. An unbiased forward genetic screening led to the identification of a novel gene, f56e10.1 (recently named as picd-1) as an important factor required for TE silencing. Loss of picd-1 function caused dysregulation of several short regulatory noncoding RNAs, de-silencing of transposons , increased DNA damage, and germ cell death. The mutant animals are fully penetrant sterile when maintained at 26.5 , and their brood size is significantly less than that of wt control animals raised at 25 . Total RNA sequencing of the RNA extracted from synchronized wt and picd-1 mutant animals of the early L4 developmental stage identified the differentially expressed genes. We have seen widespread dysregulation of several classes of repeat sequences and TEs. Small RNA sequencing from these mutant animals revealed that a subset of 22G RNAs is depleted. Additionally, the picd-1 mutant animals revealed significant DNA damage as revealed by anti-RAD-51 antibody staining. Our results indicate that the loss of picd-1 causes dysregulation of siRNAs, de-silencing of TEs and repeat elements, and increased DNA damage, all of which contribute to serious reproductive abnormalities. I propose to fully comprehend picd-1 function/s and decipher its mechanism of action in TE and repeat silencing and genome maintenance.

POSTER NUMBER: 17

NODULIN HOMEOBOX is required for heterochromatin homeostasis in Arabidopsis

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Arabidopsis NODULIN HOMEOBOX (NDX) is a nuclear protein described as a regulator of specific euchromatic genes within transcriptionally active chro- mosome arms. Here we show that NDX is primarily a heterochromatin regulator that functions in pericentromeric regions to control siRNA production and non-CG methylation. Most NDX binding sites coincide with pericentromeric het-siRNA loci that mediate transposon silencing, and are antagonistic with R-loop structures that are prevalent in euchromatic chromosomal arms. Inactivation of NDX leads to differential siRNA accumulation and DNA methylation, of which CHH/CHG hypomethylation colocalizes with NDX binding sites. Hi-C analysis shows significant chromatin structural changes in the ndx mutant, with decreased intrachromosomal interactions at pericentromeres where NDX is enriched in wild-type plants, and increased inter-chromosomal contacts between KNOT-forming regions, similar to those observed in DNA methylation mutants. We conclude that NDX is a key regulator of heterochromatin that is functionally coupled to het-siRNA loci and non-CG DNA methylation pathways.

POSTER NUMBER: 18

N6-Adenosine Methylation and a Reduced Level of m3G/TMG or N1-methyladenosine in RNA Appeared at UV-Microirradiated DNA Lesions

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The DNA damage response is mediated by both DNA repair proteins and epigenetic markers. We observed that N6-methyladenosine (m6A), a mark of the epitranscriptome, was abundant in RNA accumulated at UV-damaged chromatin. Significantly, inhibitors of RNA polymerases I and II did not affect the m6A RNA level at the irradiated genome, but PARP inhibitor, Olaparib, prevented m6A RNA as well as XRCC1 accumulation at UV-damaged chromatin. On the other hand, DNA damage did not change the levels of METTL3 and METTL14 methyltransferases. Only using an inhibitor of the METTL3 methyltransferase, we observed that the appearance of m6A RNA at DNA lesions is, to some extent, reduced. To this fact, in a subset of irradiated cells, the METTL16 protein, responsible for m6A in non-coding RNAs as well as for splicing regulation, was recruited to microirradiated sites. This event was additionally accompanied by radiation-induced depletion of 2,2,7-methylguanosine (m3G/TMG) in RNA. Moreover, UV irradiation also decreases the global cellular level of N1-methyladenosine (m1A) in RNA. Based on these results, we prefer a model of non-canonical m6A RNA-mediated DNA damage response, in which m6A RNA rapidly responds to radiation-induced stress and diffuses to damaged sites. This process is PARP-dependent and linked to BER (the Base Excision Repair) proteins, including XRCC1.

POSTER NUMBER: 19

Systematic profiling and kinetic modeling of uridylation reveals molecular principles of RNA quality control in Drosophila

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Uridylation-mediated RNA decay involves the non-templated addition of uridines to the 3 -end of an RNA which acts as a mark for subsequent degradation. In Drosophila melanogaster, RNA uridylation-mediated decay features the terminal RNA uridylation-mediated processing (TRUMP) complex which consists of the terminal uridylyltransferase Tailor and the uridylation-triggered exoribonuclease Dis3l2. Tailor and Dis3l2 functionally cooperate in the degradation of structured non-coding RNA in a cytoplasmic RNA quality control pathway that is conserved also in mammals. However, the molecular basis underlying targeting specificity as well as hand-over of uridylated substrates from Tailor to Dis3l2 remain poorly understood.

Here, we employ high-throughput sequencing-compatible biochemical assays to examine (1) RNA substrate binding by Tailor and Dis3l2, (2) Tailor-directed uridylation kinetics, (3) Dis3l2-mediated degradation kinetics and (4) simultaneous uridylation and decay by the TRUMP complex in the context of an array of randomized RNA substrates. We find that substrate selectivity is dictated by two complementary features of Tailor: An increased affinity to dsRNA as well as an enzymatic preference defined by the nucleotide identity at target RNA 3´-ends. Kinetic modeling revealed that the distributive uridylation activity of Tailor is modulated by oligo(U) tail length, resulting in a stereotypical RNA oligouridylation pattern that is compatible with efficient binding and enhanced decay kinetics observed for Dis3l2. Our data suggest that modulations in uridylation kinetics together with a specific binding of U-rich substrates by Dis3l2 prompts the efficient uridylation-triggered RNA decay.

Our findings reveal the molecular and kinetic details of a hand-over mechanism from Tailor (uridylation) to Dis3l2 (decay) within the TRUMP complex and provide fundamental insights into the regulation of gene expression by 3´-terminal RNA uridylation.

POSTER NUMBER: 20

A novel role for DDX52? Investigating dual functionality within a putative RNA helicase.

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Crucial molecular events in RNA processing during the cell cycle are dependent on the DExD-box family of proteins, but the molecular mechanisms of a number of these proteins are poorly understand. In humans, genetic data indicates DDX proteins are prognostic markers of cancer, with a number having already been identified for their potential therapeutic potential. We have made advances in identifying the biochemistry of DDX52, that are relevant to its association with liver and colorectal cancers. We report biochemical activities of purified human DDX52 protein in vitro, including dual actions as an ATP-dependent helicase and ATP-independent annealase of duplex DNA and DNA:RNA hybrids. We also report cellular phenotypes from CRISPR-Cas9 based genetic editing of DDX52 within human cells. This begins to delineate specific roles for DDX proteins that are of relevance to genome stability and the cell cycle.

POSTER NUMBER: 21

Trimeric SOSS1 complex promotes transcription at double-strand breaks

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The most toxic forms of DNA damage are the DNA double-stranded breaks (DSBs), whose efficient and timely repair requires transcription. How the transcription cycle at DSBs is regulated remains unknown. Here, we show that hSSB1 is phosphorylated and recruited to DSBs by damage-activated tyrosine kinase c-Abl, where it associates with INTS3 and c9orf80 to form trimeric SOSS1 complex. Furthermore, hSSB1 binds to R-loop structures formed at DSBs and formation of trimeric SOSS1 complex is required to suppress the inhibitory effect of RPA in hSSB1-mediated binding to R-loops. Finally, trimeric SOSS1 promotes liquid-liquid phase separation. The phase-separated SOSS1 complex then serves as a scaffold for phosphorylated RNAPII C-terminal domain (CTD) to stimulate transcription. Loss of trimeric SOSS1 leads to delay in gH2AX clearance and inhibition of DNA repair pathways, which underpins its biological importance in the context of RNA dependent DNA damage response.

POSTER NUMBER: 22

Determination of genes involved in the miRNA turnover in Arabidopsis thaliana

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microRNAs (miRNAs) are a class of small noncoding RNAs of 21 to 24 nucleotides long found in both plants and animals. They play important roles in the growth, the immune system, and the stress response of plants by reducing the level of messenger RNAs (mRNAs) they target. As their role is crucial for the survivability of the organism, miRNA levels need to be tightly regulated in cells by modulating their degradation rate - either by differential protection with 3'-2'-O-methylation, or by upregulation of degraders, or by regulation of their production. Among the different pathways leading to their degradation, tailing and truncation are thought to be the main mechanism of their turnover. Tailing occurs by the addition of Us at the 3' end of the miRNA by cooperative enzymes HESO1 and URT1 while the protein SDN1 is responsible for the truncation of non urydilated miRNAs. However, the protein responsible for the degradation of U-tailed miRNAs is still unknown. After systematic crossing of 6 different HESO1 interactor SALK mutants with a hen1 background, we focused on the splicing factor SUA (suppressor of abi3-5) which partially rescued the phenotype of the hen1 background, as well as northern blots result also showed a rescue of the level of some miRNAs. mRNA sequencing analysis suggest that SUA may alternatively splice genes involved in miRNA turnover, with HESO1 and an RNAse of interest being downregulated in sua compared to the wild type. Together, these results are indicative that either alternative splicing of HESO1 by SUA may regulate miRNA steady-state level and/or that the RNAse of interest may be responsible for the degradation of U-tailed miRNAs. Confirmation of the downregulation of those genes by western blots and the rescue of miRNAs' level by sRNA sequencing will follow.

POSTER NUMBER: 23

Transposon silencing in Spirodela polyrhiza, for whom size matters.

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Transposons (TEs) are mobile DNA sequences capable of colonizing their host and driving important evolutionary changes. Yet, in the short term, their mobilization is associated with loss-of-function mutations. To ensure genome stability, multi-layered processes secure their repression through the formation of heterochromatin. In plants, heterochromatin is characterized by a high level of DNA methylation (5mC), repressive histone post-translational modifications (HPTMs) and a specific histone variant. Because of the interdependence of these pathways, assessing the importance of each layer remains challenging. To address this point, we investigate a family of flowering plants (duckweeds) that do not present an active the central pathway responsible for the deposition of DNA methylation, RNA-directed DNA methylation (RdDM). RdDM relies on the generation of TE specific 24nt small-interfering RNAs (siRNAs). Our data from the most basal specie (Spirodela polyrhiza) depict a binary behaviour, at the whole organism level. In absence of RdDM activity, including both transcription and siRNA production levels, and more generally in an siRNA-independent manner, 5mC patterns are established over recent TE insertions along with the repressive HPTM H3K9me, while old insertions are devoid of any

repressive marks. These results suggest that siRNAs could only be required for silencing establishment or that Spirodela polyrhiza uses an alternative pathway for de novo 5mC over TE. In addition, unlike other flowering plants (e.g., Arabidopsis), duckweeds in most cases present a single copy of the gene involved in silencing. This represents a unique opportunity to study the recent evolution of silencing pathways in flowering plants. To this end, we are currently developing transgenic lines for key regulators of both 5mC and HPTM pathways (i.e., DDM1, DRM2, MET1, CMT3, SUVH4/5/6).

POSTER NUMBER: 24

A direct protein-protein interaction network of cytoplasmic Drosophila piRNA pathway factors

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PIWI-interacting RNAs (piRNAs) are small, non-coding RNAs that play a crucial role in maintaining genome integrity and fertility in animals. They are specifically expressed in the gonads of animals, where they form complexes with PIWI proteins and mediate the silencing of retrotransposons and other genes at both transcriptional and post-transcriptional levels. While numerous proteins have been identified to be essential for piRNA biogenesis and function in Drosophila their functions at the molecular and mechanistic levels remain largely unknown.

We aimed to identify and characterize direct interactions between piRNA pathway factors. However, one of the main challenges in studying these proteins using conventional biochemical methods is their structural complexity, with many factors being large multidomain proteins that also contain a substantial degree of predicted disorder. To overcome this challenge, we developed a simple and quick cell culture-based protein-protein interaction assay called ReLo, which specifically detects direct interactions. Using ReLo, we screened pairwise interactions between 25 cytoplasmic and mitochondrial proteins, confirming all previously reported interactions based on crystal structures, as well as several interactions identified using co-immunoprecipitation experiments. In addition, we identified a couple of novel interactions. To characterize confirmed and novel interactions at the molecular level, we used a combination of ReLo-based assays, in vitro binding studies, and structural modeling using AlphaFold2-multimer. Here, we present data that shed light on the complexes formed by the mitochondrial proteins Gasz and Daedalus, and by the TDRD Vreteno and the Yb/TDRD12-related DEAD-box RNA helicases BoYb and SoYb. We also present data describing a multisubunit complex consisting of Tejas (TDRD5), RNA helicases Vasa (DDX4) and Spindle-E (TDRD9), as well as either Maelstrom or the TDRD Krimper. Together, we believe that knowledge about direct interactions between essential Drosophila piRNA pathway factors will likely pave the way for future research on the molecular mechanisms underlying piRNA biology.

POSTER NUMBER: 25

Downregulation of miR-21 and CORO1C in glioblastoma cells upon treatment with a new carbonyl compound

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Glioblastoma multiforme (GBM) is considered the most malignant brain tumour accounting for five-year survival rates of only 6.4%. Despite current efforts in clinical research aiming to improve patients' survival, the prognosis remains poor. Beta-amino carbonyl compounds are a class of synthetic molecules with potential anti-cancerous properties that might provide an alternative management strategy for patients. We aimed to investigate the action of a new beta-amino carbonyl compound, N-(4-Benzyloxybenzylidene)-2-bromo-4-methylaniline (SHG-8), and its effect upon GBM cells via several in vitro assays.

In silico analysis was performed to identify the most deregulated miRNAs and their mRNA targets in GBM. Quantitative real-time polymerase chain reaction (RT-qPCR) was carried out to verify the expression profiles of selected miRNAs and their mRNA targets. In vitro assays including MTT, cellular ROS, colony forming and wound healing were performed to assess the effects of SHG-8 in U87MG cells prior- and post-exposure. Small RNA-sequencing was performed to identify the miRNAs that SHG-8 compound activates or supresses.

Bioinformatics analysis revealed that miR-21 was overexpressed in GBM and has a direct involvement in the Wnt/beta-catenin pathway, as does CORO1C. CORO1C was tested and found significantly upregulated in GBM and downregulated in GBM treated cells. MTT demonstrated an IC50 of 40μM. Significant release of reactive oxygen species was observed following 30 minutes incubation with 50μM of SHG-8 and 90 minutes incubation with 100μM of SHG-8. The colony formation and wound healing assays showed significantly reduced cell migration within U87MG cells. sRNA-seq results showed that miR-21 was significantly downregulated in the presence of the SHG-8 compound. RT-qPCR results showed that CORO1C was significantly downregulated within treated cells. Synthetic molecules provide a future, opportunistic approach for the management and potential treatment of GBM. The beta-amino carbonyl compound, SGH-8, has shown promising in vitro results, but additional studies are necessitated to verify the findings.

POSTER NUMBER: 26

New beta-amino ketone compound regulates cell death in vitro and upregulates miR-6715b-3p in colon cancer cells

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Colorectal cancer (CRC) is one of the most prevalent and lethal cancers worldwide. Currently approved checkpoint inhibitors for CRC treatment targeting CTLA-4, PD-1 and PD-L1 have shown resistance. To tackle this issue, new therapeutics are targeting an emerging immune checkpoint protein known as Siglec-15 through blocking antibodies. Siglec-15 is associated with CRC progression due to its role in promoting immune suppression. However, it remains unclear how SIGLEC15 expression is regulated in CRC cells by microRNAs

(miRNAs). Herein, the effectiveness of the N-(4-Benzyloxybenzylidene)-2-bromo-4-methylaniline (SHG8) compound was assessed in SW480 CRC cells. In silico analysis explored the expression of selected miRNAs involved in regulating SIGLEC15 expression. Small RNA-sequencing (sRNA-seq) and real time quantitative polymerase chain reaction (RT-qPCR) methods were used to screen the expression of predicted miRNA mediated SIGLEC15 expression pre- and post-SHG8 treatment. SHG8 showed a significant cytotoxic effect on the CRC cell line viability, migration, and colony formation in a dose-dependent manner with an IC50 value of ~20µM. SIGLEC15 gene and protein expression was reduced following SHG8 treatment. In silico analyses predicted several miRNAs involved in the regulation of SIGLEC15, which were also confirmed by sRNA-seq analysis. The latter also revealed that miR-6715b-3p was the most up-regulated miRNA when treated with SHG8. SHG8 has demonstrated its potential as a therapeutic treatment for CRC. By downregulating SIGLEC15 expression, it may provide an alternative in treating Siglec-15 positive CRC tumours. Notably, Siglec-15 is expressed on mutually exclusive populations of cancer cells with respect to PD-L1. Therefore, this study could pave the way for combination therapies with PD-L1 and Siglec-15 antagonists.

POSTER NUMBER: 27

ATPase-dependent maturation of 60S pre-ribosomes

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Biogenesis intermediates of nucleolar ribosomal 60S precursor particles undergo a number of maturation steps before they transit to the nucleoplasm and are finally exported into the cytoplasm. The huge AAA+-ATPase Rea1 promotes two essential consecutive maturation steps on evolving pre-60S assembly intermediates. First, Rea1 participates in nucleolar exit by releasing the Ytm1–Erb1 heterodimer. Later it releases assembly factor Rsa4, priming the pre-60S particles for nuclear export. Here, we show that a highly conserved motif within the C-terminal domain of Rea1 facilitates both nuclear import of the protein and, in a second function, ligand extraction by promoting intramolecular docking of the C-domain onto the N-terminal AAA+ motor domain. Furthermore, we unveil how the DEAD-box RNA helicase Spb4 is integrated into the first Rea1-dependent remodelling event. Spb4 binds to a specific class of late nucleolar pre-60S intermediates, whose cryo-EM structure revealed how the helicase facilitates melting of 25S rRNA helices H62/H63 prior to Ytm1–Erb1 release. In vitro maturation of such Spb4-enriched pre-60S particles, incubated with purified Rea1 and its associated pentameric Rix1-complex in the presence of ATP, combined with cryo-EM analysis depicted the details of the Rea1-dependent large-scale pre-ribosomal remodelling. Our structural insights unveil how the Rea1 ATPase and Spb4 helicase remodel late nucleolar pre-60S particles by rRNA restructuring and dismantling of a network of several ribosomal assembly factors.

POSTER NUMBER: 28

MicroRNA biogenesis differentially affected by DGCR8 knockdown in lymphoma cells

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MicroRNAs (miRNAs) are small, non-coding RNAs that inhibit gene expression at the post-transcriptional level. miRNAs are transcribed as primary miRNAs (pri-miRNAs) that are processed by Drosha-DGCR8 complex to precursor miRNA (pre-miRNA) and exported to the cytoplasm to be processed by Dicer to mature miRNAs. In this study, we aim to determine the effect of DGCR8 inhibition on miRNA biogenesis. We used two short hairpin RNAs, shDGCR8-1 and shDGCR8-2, in lentiviral vectors to inhibit DGCR8 expression in Hodgkin lymphoma L1236. We showed that the levels of DGCR8 protein were significantly decreased upon shDGCR8-1 and shDGCR8-2 in L1236 compared to scrambled negative control (SCR). To analyze levels of pri-miRNAs upon DGCR8 knockdown, we performed RNA-seq for L1236 cells transduced with shDGCR8-1 or SCR. We showed that the levels of 154 out of 726 pri-miRNAs were increased upon shDGCR8-1 compared to SCR. Next, we validated the results for selected miRNAs and pri-miRNAs with qRT-PCR. The levels of miR-155, miR-19b and miR-378a decreased, whereas the levels of the pri-miR-155 and pri-miR-17~92 (pri-miR-19b) increased upon shDGCR8 compared to SCR in L1236 cells. Additionally, we showed that DGCR8 knockdown induced L1236 cell death as the percentage of apoptotic cells increased up to 2 folds for L1236 cells with shDGCR8-1 compared to SCR. We also analyzed cell cycle distribution and showed up to 4 fold increase in the percentage of L1236 cells in G1/G0 phase for shDGCR8-1, also upon 8Gy dose of irradiation, compared to SCR. In conclusion, we showed that not all miRNAs were equally affected by DGCR8 knockdown and inhibition of DGCR8 induced apoptosis in L1236 cells. Further studies that involve analysis of additional miRNAs and pri-miRNAs using qRT-PCR are ongoing.
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POSTER NUMBER: 29

STUDY OF SERUM PIRNA-54265 AS A NON-INVASIVE BIOMARKER FOR COLORECTAL CANCER IN EGYPTIAN PATIENTS

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Colorectal cancer (CRC) is one of the most prevalent types of cancer worldwide. With 35% of patients under the age of 40, Egypt has the highest rate of early CRC. Early CRC is frequently asymptomatic. Most patients are diagnosed after the tumor has invaded and spread. PIWI-interacting RNAs (piRNAs) are a new class of small non-coding RNAs with little-known expression patterns in tumors. Several piRNAs have been identified as oncogenes in CRC. It was discovered that piRNA-54265 increased CRC cell proliferation

and invasiveness via the STAT3 pathway. Our goal was to determine if serum piRNA-54265 expression levels could be used as a non-invasive CRC biomarker and how it compares to standard biomarkers (CEA & CA19-9). Using stem-loop quantitative reverse transcriptase PCR, we determined the expression levels of piR-54265 in 40 serum samples from Egyptian CRC patients and 30 serum samples from healthy controls. Serum piRNA-54265 levels in CRC patients were significantly higher than in controls. It was also significantly higher in stages III and IV than in earlier stages (I and II). Our research found that serum piRNA-54265 could distinguish early CRC from controls. The receiver operating characteristic curve (ROC curve) test showed serum piRNA-54265 had 94.4% sensitivity and 100 % specificity in detecting early-stage CRC, with an area under the curve of 0.991, outperforming both CEA and CA19-9. These results show that piRNA-54265 expression increases in the serum of CRC patients in a stage-dependent manner. It may also be used as an early non-invasive diagnostic biomarker for CRC.

POSTER NUMBER: 30

Oxford Nanopore sequencing reveals changes in ribosomal RNA modifications in a cellular skin aging model

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The degree of variability in ribosomal RNA modifications throughout the human life span is currently unknown, although there is evidence of many substoichiometrically modified sites. One hallmark of human aging is the accumulation of senescent cells, which have a detrimental impact on the surrounding healthy tissue and contribute to age-associated diseases by eliciting a senescence-associated secretory phenotype (SASP). Considering the major physiological changes in senescent cells, the evidence of an adapting translatome and the correlation of rRNA modifications with lifespan in model organisms, rRNA modifications might change as well in cellular senescence.
To study potential modification changes in cellular senescence, we exposed normal human keratinocytes to UVB radiation and environmental pollutants in the form of diesel particle matter (DPM). This treatment mimics the environmental factors accelerating aging and inducing senescence, to which the human skin is heavily exposed. To determine changes in the epitranscriptome at known rRNA modification sites between treated and control human keratinocytes, Oxford Nanopore direct RNA sequencing targeting rRNA was used. As additional control we utilized paraquat, which robustly induces cellular senescence in human keratinocytes. Preliminary results show subtle changes at pseudouridylated and 2'-O-methylated rRNA sites, for most of which varying levels of modification have been previously reported. The validation of altered levels of 2-O-methylated or pseudouridylated sites with alternative methods is currently in progress.
Together, these data will shed light on the variability of rRNA modification sites in treatment-induced cellular senescence in human keratinocytes. This will be important for a better understanding of the impact of environmental factors like radiation and pollutants on our body's largest organ, the skin. Moreover, our study will also aid the better characterization of senescent cells, which accumulate in our body throughout aging.

POSTER NUMBER: 31

Upa1 and Upa2 are part of an unconventional snoRNP

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Early pre-60S particles are among the least understood maturation intermediates in ribosome biogenesis. An entry point into a deeper and better understanding of these early maturation steps could be the characterization of novel so far unrecognized ribosome biogenesis factors that function during early pre-60S assembly.
We identified two novel ribosome biogenesis factors Ymr310c and Ygr283c (recently renamed into Upa2 and Upa1, respectively), which are so far uncharacterized proteins. Upa1 and Upa2 are two novel potential methyltransferases belonging to the SPOUT family. Together, Upa1 and Upa2 can form a heterodimer that interacts both physically and genetically with the Npa1 complex, an important actor during early stages of pre-60S maturation. Moreover, we found that Upa1 and Upa2 bind to the snR37 H/ACA-type snoRNP and we could show that a deletion of Upa1 and Upa2 leads to alterations in the composition of pre-60S particles. Our results suggest that snR37 is an unconventional snoRNP containing in addition to the H/ACA core proteins also Upa1 and Upa2 as stable components. We propose that the snR37 complex bridges RNA and protein elements within early pre-60S particles, thereby acting as a scaffold for rRNA folding.

POSTER NUMBER: 32

ELAV mediates circular RNA biogenesis in Drosophila neurons

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Circular RNAs (circRNAs) are single-stranded RNA molecules covalently closed into a continuous loop structure that results from the back-splicing of precursor messenger RNAs (pre-mRNAs). circRNAs are primarily present in the nervous system of animals, and their expression significantly increases with age. Moreover, circRNAs play a critical role in regulating various aspects of neural physiology, including synaptic plasticity, neuronal differentiation, and synaptic transmission. Despite their importance, the mechanisms underlying circRNA regulation in the nervous system remain poorly understood.
In this study, we demonstrate the role of pan-neuronal RNA-binding protein ELAV in establishing the neuronal circular RNA landscape. Across the animal kingdom, ELAV constitutes an indispensable factor in the maintenance of neuronal identity and development of the nervous system. By regulating alternative splicing (AS) and alternative polyadenylation (APA), ELAV determines specific molecular signatures that characterize neural identity. Here, we investigated the impact of ELAV loss-of-function on circular RNAs in Drosophila neurons. Deep-sequencing total RNAs from flow-sorted cell populations in developing embryos, including elav mutant neurons, we show that ELAV loss results in a widespread depletion of neuronal circRNAs. RNA Immunoprecipitation and sequencing revealed that ELAV binds to pre-mRNAs of virtually all target circRNA host genes, without binding to the mature circRNA itself. Our findings suggest that ELAV maintains the balance between the production of protein-coding mRNA isoforms and circular RNA formation, highlighting its pivotal role in regulating neuronal RNA homeostasis.

POSTER NUMBER: 33

Human senataxin is a bona fide R-loop resolving enzyme and transcription termination factor

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Prolonged pausing of the transcription machinery may lead to the formation of three-stranded nucleic acid structures, called R-loops, typically resulting from the annealing of the nascent RNA with the template DNA. Unscheduled persistence of R-loops and RNA polymerases may interfere with transcription itself and other essential processes such as DNA replication and repair. Senataxin (SETX) is a putative helicase, mutated in two neurodegenerative disorders, which has been implicated in the control of R-loop accumulation and in transcription termination. However, understanding the precise role of SETX in these processes has been precluded by the absence of a direct characterisation of SETX biochemical activities. Here, we purify and characterise the helicase domain of SETX in parallel with its yeast orthologue, Sen1. Importantly, we show that SETX is a bona fide helicase with the ability to resolve R-loops. Furthermore, SETX has retained the transcription termination activity of Sen1 but functions in a species-specific manner. Finally, subsequent characterisation of two SETX variants harbouring disease-associated mutations shed light into the effect of such mutations on SETX folding and biochemical properties. Altogether, these results broaden our understanding of SETX function in gene expression and the maintenance of genome integrity and provide clues to elucidate the molecular basis of SETX-associated neurodegenerative diseases.

POSTER NUMBER: 34

Fast evolution of piRNA cluster expression in mouse

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Piwi-interacting RNAs (piRNAs) are small non-coding RNAs responsible for the silencing of transposable elements in the germline of most animals. They are produced from long single-stranded transcripts that derive from discrete genomic loci called piRNA clusters. piRNAs and piRNA clusters are highly diverged between species showing almost no evidence of selection constraint. Considering their fast turnover, we wondered how the expression of piRNA clusters evolves in short evolutionary time scales. To address this, we focused on differences in postnatal piRNA expression in different inbred strains of mice and closely related murine species. We found significant differences in piRNA clusters within and across species. Within Mus musculus, we found that clusters with polymorphic endogenous retroviruses were overrepresented among those with highly variable piRNA cluster expression. Comparing closely related murine species that shared a common ancestor six million years ago, we found high divergence in the expression of piRNA clusters. We considered the effect of features previously associated with piRNA-producing protein-coding genes in the turnover of piRNA clusters. Although genes with long untranslated regions (UTR) and long first exons are significantly associated with piRNA production, we found no evidence that changes in UTR or exon length lead to changes in piRNA production between murine species. We found that the best predictor of piRNA cluster expression conservation is piRNA cluster expression level. Taken together our results reveal that young endogenous retroviruses are potent drivers of piRNA cluster gains and that piRNA abundance is linked to piRNA evolution.

POSTER NUMBER: 35

Deep sequencing of circulating microRNAs in pediatric patients with juvenile and adolescent idiopathic scoliosis

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Scoliosis is one of the most frequent spinal deformities and is predominantly found among children under 13 years old. 80% of cases are of unknown origin and idiopathic scoliosis is considered a multifactorial disease. Currently, there are no clinical biomarkers of the progression of idiopathic scoliosis available. Such biomarkers could be circulating microRNAs. microRNA expression levels were previously shown to be altered under various pathologic conditions. This study aims to evaluate the prognostic potential of circulating microRNAs in idiopathic scoliosis.

In a prospective mono-centric study carried at University Hospital Brno, blood plasma samples from 24 patients (12 with progression, 12 without progression) diagnosed with juvenile or adolescent idiopathic scoliosis and 24 healthy controls were included. Blood plasma samples from patients were collected at four time points. Total RNA was isolated using miRNeasy Serum/Plasma kit (QIAGEN, USA). cDNA libraries were prepared using Qiaseq miRNA UDI Library Kit (QIAGEN, USA). The sequencing analysis was performed using NovaSeq 6000 S1 v1.5 Kit - 100 cycles using the NovaSeq 6000 instrument (Illumina). After preprocessing of sequencing data, reads were mapped against database miRBase v 22 using the miraligner tool v 3.2. Obtained data were statistically evaluated in R environment v 4.0.4. Differential expression analysis was performed using the DESeq2 package v 1.30.1.

When miRNA profiles of samples from patients with and without progression were compared 26 miRNAs ($P<0,05$) were found to have significantly deregulated expression levels between the groups. 18 miRNAs were upregulated and 8 miRNAs were downregulated in idiopathic scoliosis patients' blood plasma ($P<0,05$). Subsequently, dynamics of miRNAs levels throughout the time points were compared between patients with and without progression.

Our findings suggest that circulating miRNAs could serve as potential biomarkers of progression in pediatric idiopathic scoliosis.

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POSTER NUMBER: 36

A splice variant of Poly(A)-specific ribonuclease in human cells

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Poly(A)-specific ribonuclease (PARN) catalyses the shortening of poly(A) tails, the first and rate-limiting step in mRNA degradation. PARN also mediates the maturation of a diverse and ever-expanding repertoire of non-coding RNAs, spanning from microRNAs and piRNAs to the telomeric RNA component. Several splice variants of PARN have been reported in RNA sequencing studies, yet, a study on the biochemical characterization or biological significance of these is still pending. Herein, we detect a splice variant of PARN, PARN-v1, in pleural malignant mesothelioma (PMM) cell lines. PARN-v1 mRNA and protein levels are increased in cells originating from all PMM subtypes, whilst it is barely detectable in benign pleural cells. Cloning of PARN-v1 mRNA from M14K epithelioid PMM cells and sequence analysis revealed that the variant results from an alternative 5′ splice site selection at the 3′ end of exon 1. The spliced-out sequence includes the start codon in exon 1 of full-length PARN (PARN), while the start codon for PARN-v1 locates in exon 4. Compared to PARN, the resulting PARN-v1 polypeptide lacks a 61-amino acid sequence from its N-terminus including two catalytic residues. Surprisingly, deadenylation assays reveal that PARN-v1 retains its poly(A)-shortening activity. Molecular modelling and site-directed mutagenesis reveal residues that shape the active site of PARN-v1 and compensate for the two missing ones of PARN. To identify factors that may regulate PARN expression, we investigate the role of several microRNAs and show that miR-29a and miR-1207 modulate the levels of PARN and PARN-v1. We find that MRC-5 lung fibroblast cells express mainly PARN-v1, while PARN is barely detectable. Finally, we silence PARN-v1 expression and analyse the impact on MRC-5 proteome. Given the role of PARN in the regulation of gene expression the investigation of the biological significance of its splice variants remains an open challenge.

POSTER NUMBER: 37

The potential role of exosomal small-RNAs in the early prediction of Preeclampsia combined with Intrauterine Growth Restriction

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Preeclampsia (PE) is a pregnancy related multisystemic syndrome, characterized by hypertension and proteinuria after the 20th week of pregnancy. Early onset PE, appearing before the 34th week of the pregnancy, is associated with placental disfunction and commonly combined with fetal intrauterine growth restriction (IUGR). Late onset PE, however, is caused by chronic underlying diseases of the mother and affects endothelial function.

Exosomes are a subtype of extracellular vesicles, which mediate cell-to-cell communication with their cargo, including various types of RNAs. The composition of the exosomal RNA cargo is unique and protected from degradation, and application of exosomal small RNAs as biomarkers has an emerging role because they are stable and detectable in various biofluids, offering a non-invasive examination method.

To examine the pathophysiological mechanism of PE combined with IUGR, we aimed to investigate the differences of plasma

exosomal small-RNA content between healthy and preeclamptic pregnancies aggravated with IUGR before the development of symptoms. We performed a high-throughput exosomal small RNA sequencing using a low volume first trimester maternal plasma samples from healthy and PE+IUGR pregnancies to find differences in small RNA expression levels. The analysis revealed differential expression in case of 13 miRNAs and 2 piRNAs in the PE+IUGR pregnancies compared to normal conditions. Interestingly only one of the piRNAs was down-regulated, whereas all the other differentially expressed small RNA species were up-regulated in the patients. To validate the sequencing data we measured the expression levels of 5 candidate small RNAs by real-time qPCR in additional exosomal samples. Results of the analyses, including the identified potential targets of the examined small RNAs will be presented in detail at the conference.

This project is implemented within the Cooperative Doctoral Programme and supported by NKFIH and ITM (Research Project Code: C1012947), and by the PC-II-12/2022 grant of the Hungarian Academy of Sciences.

POSTER NUMBER: 38

DNA demethylation causes expression of tumor suppressive instead of oncogenic p63 isoform in squamous cell carcinoma.

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The TP63 gene encodes two major protein isoforms; TAp63 contains a p53-like transcription domain and consequently has tumor suppressor activities whereas ΔNp63 lacks this domain and acts as an oncogene (1). These two variants are known to show distinct expression patterns in normal tissues and tumors, but the mechanisms involved in their regulation are poorly understood. In squamous epithelial cells with high levels of ΔNp63 and low/undetectable TAp63, the DNA demethylating agent decitabine caused a dose-dependent increase in TAp63, with a simultaneous reduction in ΔNp63, indicating DNA methylation-dependent regulation at the isoform-specific promoters. The reduction was also observed in the direct transcriptional target of ΔNp63, the basal cytokeratin KRT5, which confirms a functional change in p63 activity after DNA demethylation. We also detected high level of methylation of three CpG sites in the TAP63 promoter in these cells, which was reduced by decitabine. DNMT1 depletion using inducible shRNAs partially replicated these effects, including an increase in the ratio of TAP63: ΔNP63 mRNAs, a reduction in ΔNp63 protein and reduced KRT5 mRNA levels. Finally, high DNA methylation levels were found at the TAP63 promoter in clinical squamous cell carcinoma samples and matched normal tissues. We conclude that DNA methylation at the TAP63 promoter normally silences transcription in squamous epithelial cells, indicating DNA methylation as a therapeutic approach to induce this tumor suppressor in cancer. While a variety of mechanisms may be involved in producing the opposite effects of DNA demethylation on TAP63 and ΔNP63, we propose an "either or" mechanism in which TAP63 transcription physically interferes with the ability to initiate transcription from the downstream ΔNP63 promoter on the same DNA strand.

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POSTER NUMBER: 39

In vitro reconstitution of mixed lineage kinase zak activation and regulation

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Precise translation initiation and elongation rates ensure multiple ribosomes can seamlessly move along the same mRNA. However, environmental insults can cause an actively translating ribosome to pause during this process and a lagging ribosome on the same transcript to collide with the stalled ribosome. These collisions activate the ribotoxic stress response (RSR) mediated by the mitogen-activated protein kinase kinase kinase (MAP3K) ZAKα, which mediates the downstream phosphorylation of the MAPKs JNK and p38 Previous in vivo studies have shown that ZAKα associates with elongating ribosomes in unstressed conditions allowing it to act as an sensor of translation stress. This interaction is likely through the highly negatively charged C-terminal ribosome binding region. Upon sensing ribosome collisions, ZAKα becomes phosphorylated and releases from the ribosome moving to the free fraction of a sucrose gradient. Here, we use in vitro assays to reconstitute ZAKα ribosome binding and activation and explore interactions with downstream factors including 14-3-3 proteins and the MAPKK substrates. Autophosphorylation assays with radiolabeled ATP indicate that the phosphorylation status of ZAKα modulates its activity in vitro independent of ribosomes. We use a series of phosphorylation and truncation mutants to refine ribosome binding domains and reveal the binding site for 14-3-3 kinase scaffold proteins. Using these in vitro assays we are developing an in vitro reconstitution of ZAK activation on collided ribosomes and downstream signaling.

POSTER NUMBER: 40

Metabolic labeling captures mRNA dynamics in response to translational stress

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Conditions that induce ribosome collisions, such as UV irradiation or low doses of translation elongation inhibitors, lead to activation of signaling pathways that dictate whether a cell survives or undergoes apoptosis. The effects of these translational stresses on the transcriptome remain poorly characterized. Here we use nucleoside recoding chemistry combined with high-throughput sequencing to investigate how ribosome collisions induced by UV irradiation or elongation inhibitors impact mRNA dynamics in human cells. As expected, our sequencing results reveal differential expression of many transcripts under both of these conditions. We find that

while collisions induced by emetine promote the transcriptional activation of a subset of transcripts, most of the observed differential expression is explained by changes in the rate of mRNA degradation, consistent with previous literature stating that mRNA decay occurs co-translationally. A majority of the stabilized mRNAs encode zinc finger proteins, which are transcripts known to have short poly-A tails. Stabilization of this subset of transcripts is explained in part by their short half-lives, but not by their presence on polysomes before or after emetine treatment. Conversely, we find that most differential expression in response to UV irradiation is driven by a general decrease in transcription. Our results reveal two different sets of mRNA dynamics in the presence of ribosome collisions. Future work will assess the molecular mechanisms dictating the observed changes in mRNA stability.

POSTER NUMBER: 41

PARP-dependent and NAT10-independent acetylation of N4-cytidine in RNA appears in UV-damaged chromatin

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RNA modifications have been known for many years, but their function has not been fully elucidated yet. For instance, the regulatory role of acetylation on N4-cytidine (ac4C) in RNA should be explored not only from the view of regulation of RNA stability and mRNA translation but also during DNA repair. Here, we observe a pronounced positivity of ac4C RNA at DNA lesions of interphase cells and in irradiated cells in telophase. Ac4C RNA appears in the damaged genome from 2 to 45 minutes after microirradiation. However, RNA cytidine acetyltransferase NAT10 did not accumulate to damaged sites, and NAT10 depletion did not affect the pronounced recruitment of ac4C RNA to DNA lesions. This process was not dependent on the G1, S, and G2 cell cycle phases. To this fact, we observed that the PARP inhibitor, olaparib, prevents the recruitment of ac4C RNA to damaged chromatin. Together, our data imply that acetylation of N4-cytidine on especially small RNAs is an important RNA modification that, with a high probability, mediates DNA damage repair. Ac4C RNA likely causes de-condensation of chromatin in the vicinity of DNA lesions accessible for other DNA repair factors playing a role in DNA damage response. Alternatively, RNA modifications, including ac4C, could be direct markers of damaged RNAs.

POSTER NUMBER: 42

piRNA-mediated transcriptional silencing of LTR-retrotransposons in Aedes mosquitoes

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Radboudumc

The piRNA pathway is a crucial for maintaining the genomic integrity by silencing transposable elements in the germline of animals. However, in Aedes mosquitoes the PIWI-gene family has expanded to seven member including Piwi4-6 and Ago3, which are also involved in piRNA biogenesis in somatic tissues. However, the individual functions of these proteins, including Piwi6, remain unclear. To investigate the function of Piwi6, we generated Piwi6 knockouts in an Aedes albopictus cell lines, and analyzed the small-RNAs repertoire. We found that the loss of Piwi6 did not drastically affect overall piRNA levels, but it did result in a significant increase in sense piRNAs derived from a specific class of Long Terminal Repeat (LTR)-retrotransposons. Furthermore, LTR-retrotransposon transcripts were upregulated upon Piwi6 loss. We therefore, hypothesized that Piwi6 controls transposable elements through transcriptional gene silencing, akin to Piwi in Drosophila melanogaster. Confirming this, we observed a decrease in the H3K9me3 mark and an increase in the Pol II occupancy on specific LTR-retrotransposons upon Piwi6 loss. Our results establish Piwi6 as an essential factor for piRNA-mediated transcriptional silencing of LTR-retrotransposons in Aedes mosquitoes, marking the first study to explore the nuclear arm of the Aedes piRNA pathway.

POSTER NUMBER: 43

Deciphering the function and regulation of dormant ribosomes

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Translation, the process by which proteins are made, is an essential cellular process but also consumes a lot of energy, requiring tight regulation. This poses a major challenge in eggs, where many organisms store large numbers of ribosomes to allow rapid protein production after fertilization. To preserve energy, eggs are therefore translationally repressed. In zebrafish and Xenopus eggs ribosomes are kept in a dormant state by a molecular network of conserved factors, which bind and block functionally important sites of the ribosome, thereby inhibiting translation and stabilizing the ribosome. The factors binding these 'dormant ribosomes' include the two paralogous proteins Dap and Dap1b, which had not previously been described to be associated with the ribosome but were found to bind to the ribosome's polypeptide exit tunnel and inhibit translation (Leesch and Lorenzo-Orts et al., 2023).
It has been shown that dormant ribosomes are important for successful embryonic development in zebrafish, however, three main questions still need to be answered to understand the regulation of dormant ribosomes and the molecular function of Dap/Dap1b: First, how are the dormancy factors released from the ribosome during embryonic development to allow the increase in translational activity? Second, what is the difference between Dap and Dap1b in their function and regulation? Third, what is the function of dormant ribosomes in mammals, and more specifically in somatic mammalian cells?
To tackle these questions, we are using in vivo and in vitro experiments in zebrafish and mouse models, including loss-of-function analyses, mass spectrometry and biochemical assays. Our research will help to understand the physiological relevance and regulation of a novel mode of ribosome storage and translational control.

POSTER NUMBER: 44

Anticancer activity of Salvia Officinalis in breast cancer

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Breast cancer is the most common invasive cancer type in middle aged and older women. reast cancer is treated in several ways, such as surgery, chemotherapy, radiation and hormonal therapy. Despite all types of treatments, breast cancer is the second leading cause of cancer death in women in the United States, following lung cancer. (ASCO). According to the American Society of Clinical Oncology (ASCO), 684,996 women worldwide died from breast cancer in 2020. Therefore, many studies are focusing on better understanding the cellular mechanisms that lead to tumor formation and cancer metastasis, in hopes for achieving higher cure rates.
JAK2 signaling is linked to certain types of cancer and influences proliferation and apoptosis, as demonstrated by abnormal STAT3 signaling in malignant cells. (Sen, M., 2015). Recent evidence suggests that JAK2/STAT3 signaling is constitutively active in the breast cancer. A Lot of efforts have been performed to target JAK2 that is associated with progression of cancer cells. in our research, we found S. Officinalis suppressed JAK2 expression and exerts strong anticancer potential in MCF7 breast cancer cells. In addition, we Investigated the cytotoxic effect of S. Officinalis on MCF7 breast cancer cells and revealed the role of S. Officinalis on the expression of JAK2, BAX, BIRC5 & Bcl-xL at mRNA levels. We also investigated the effect of miR-216a-5p on the JAK2 mRNA expression and downstream targets, BAX, BIRC5 & Bcl-xL.

POSTER NUMBER: 45

EU incorporation-based screening reveals several novel transcription regulators

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The efficient transcription of genes requires a tight regulation of the RNA Polymerase II (RNAPII) at each step. This regulation of the RNAPII is orchestrated via its dynamic phosphorylation and a stage-specific interaction with a multitude of proteins. The direct roles of many of these proteins on the productive functioning of RNAPII is unknown.
In a quantitative label-free mass spectrometric analysis of the RNAPII associated complexes, we identified 101 strongly interacting proteins (Baluapuri A, Hofstetter J et al., Mol. Cell 2019). Among these were the canonical and well-studied proteins of Integrator complex, Mediator complex, and known transcription factors such as SPT5, SPT6 and the PAF complex. Interestingly, we identified several proteins that had a studied role in mRNA splicing, as well as some proteins which had no described roles in transcription.
In this study, to assess if these splicing and unknown proteins directly influence transcription, we performed siRNA-based high content imaging screens that utilized 5' ethynyl-uridine (EU) incorporation to assess transcriptional activity. We utilized positive and negative siRNA controls to measure the impact of the target depletion on transcription. The results of the screen revealed that several of the previously uncharacterized proteins had a strong impact on the rate of EU incorporation when depleted. However, it is known that the depletion of proteins with an siRNA pool could have several caveats, such as off-target and compensatory effects, eventually resulting in false positives. Therefore, to further strengthen these results, we used the auxin-inducible-degron (AID) system to acutely and specifically deplete selected proteins.
Currently, we are characterizing the primary effects of the depletion of these proteins on RNAPII transcription using next generation sequencing approaches to assess RNA synthesis and chromatin occupancy of RNAPII. This work will help to gain insights into new direct regulators of RNAPII transcription activity and further decipher the role of RNAPII's dynamic interactome.

POSTER NUMBER: 46

Reduction of the CRISPR-Cas mediated immunity by anti-cas transcript in Escherichia coli

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The CRISPR-Cas adaptive immune system protects many bacteria and most archaea from invading DNA. The Type I-E CRISPR-Cas system in E. coli consists of 8 genes organised into a single operon containing the casABCDE12 genes, the separate cas3 gene, and the CRISPR-1 array. Under normal laboratory growth conditions, the CRISPR-Cas system is silenced by the global repressor H- NS. However, in cells lacking H-NS, Cas3 becomes a limiting factor for CRISPR-Cas immunity. Cas3 nuclease-helicase is involved in the final step of CRISPR-Cas immunity – cleavage of invading DNA. Control of the Cas3 nuclease-helicase activity is exerted at the transcriptional and post-translational levels and by the temperature of incubation. We recently found that the structure of the Cas3 protein is altered by temperature, which causes loss of Cas3 nuclease function via a gate formed of Trp-406 opening and closing access of ssDNA to the nuclease active site. Interestingly, a short anti-cas transcript of 373 nt was identified that overlaps with the last 205 nt of the cas3 gene. It is expressed from the divergently oriented anti-Pcas promoter and is also controlled by the H-NS protein. Because this transcript can fold into an intricate secondary structure, it has been suggested that it might interact with the Cas3 protein to inhibit its activity, or anneal to the cas3 RNA transcript and inhibit its transcription termination and/or translation. Here, we show that the anti-cas transcript reduces the activity of the Cas3 protein, but the exact mechanism remains unknown.

POSTER NUMBER: 47

Design and Characterization of Flexible Nucleic Acids for Disrupting Viral Counter-Defense Machinery

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Plant viruses are a serious threat to agriculture and can cause outbreaks of viral diseases that result in food shortages and have negative impacts on human health. To defend against viruses, plants have developed mechanisms such as siRNA-mediated RNA-induced silencing, which degrades viral mRNA. However, some viruses have evolved countermeasures, such as encoding RNA silencing suppressor proteins (for example, the p19 protein from the Tombusviridae family), which can interfere with siRNA-mediated silencing and allow the virus to proliferate in plants. The p19 protein works by binding to plant siRNA and preventing RNA-induced silencing complex formation, ultimately leading to virus invasion in the plant. In this study, we designed modified nucleic acid-based molecules to inhibit the p19 protein and used molecular dynamics simulations to evaluate their efficacy. Some designed modified nucleic acids showed enhanced p19 binding compared to the control cognate binder, suggesting their potential as p19 inhibitors for crop protection.

POSTER NUMBER: 48

Specificity of 3'UTR-mediated regulatory partners in disease-related mRNAs across different cell lines

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The 3' untranslated regions of messenger RNAs (3'UTR of mRNAs) are essential in regulating various mRNA-dependent processes. 1 One of their crucial roles is post-transcriptional regulation, which controls the translation, stability, and localization of mRNAs. Post-transcriptional regulation can be categorized into three groups: A) mechanisms that repress translation, B) mechanisms that degrade mRNAs, and C) mechanisms that localize RNAs.2 miRNA machinery and proteins involved in signaling pathways possibly control these processes, which can be common or specific to certain stages of development and cell types.3
There is currently limited information about the diversity of 3'UTR-mediated regulatory partners and whether they are common or specific to individual genes or cell lines. Our study examines the factors involved in post-transcriptional regulation through 3'UTRs of both oncogenic and non-oncogenic genes associated with disease regulation. The main objective is to determine whether the differences are directly caused by a specific RNA-binding protein (RBP) or through microRNA-dependent regulation. To achieve this, we started examining the differences in translation rates influenced by selected 3'UTRs in three cell lines: HeLa, HEK293, and K562. Based on our preliminary studies, we have observed a considerable difference in translation rates resulting from the MYC 3'UTR in both HeLa and HEK cells. Next, we conducted biotin-based RNA pulldown experiments, targeting the MYC 3'UTR to compare its interactome—this way, we plan to identify the potential factors involved in mRNA 3'UTR-dependant regulation.
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POSTER NUMBER: 49

LIN-28 as a conserved factor controlling the onset of animal puberty

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The transition from juvenile to sexually mature state, known in mammals as puberty, is a fundamental feature of animal development. In the C. elegans, this transition occurs after four larval stages under the control of the heterochronic pathway, an inhibitory gene cascade consisting of two parallel arms. We aim to understand how the activities of two arms are coordinated to ensure synchronous development of different body parts and, thereby, a smooth entry into adulthood. We hypothesize that the coordinating factor is the conserved RNA-binding protein LIN-28. To uncover the mechanisms by which LIN-28 orchestrates development, we aim to characterize the molecular interaction with its two established targets, lin-46 mRNA and let-7 miRNA. We mutated the putative LIN-28 binding site in lin-46 5'UTR and let 7 primary transcript and analyzed the effect on their expression. While the mutation had no effect on mature let-7 miRNA levels, the analogous mutation introduced in lin-46 transcript resulted in precocious accumulation of LIN-46 protein. To further understand how LIN-28 represses its targets, we performed an IP-MS experiment to identify its potential co-factors. Among the interacting partners of LIN-28 we identified some of the RNA-binding proteins previously shown to interact with the mammalian LIN28, such as SSB-1 (homolog of SSB), LARP-1 (LARP1) or FIB-1 (FBL). Currently we are screening the interacting partners for causing developmental phenotypes upon RNAi mediated knock-down to further assess the functional relevance of their interaction with LIN-28.

POSTER NUMBER: 50

Unravelling the catalytic activities of Human DDX49

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DNA box helicases control RNA metabolism during transcription and translation. Among them, DEAD-box helicase 49 (DDX49) exhibits ATP-dependent RNA helicase activity and regulates mRNA export and 47s rRNA transcription in ribosome biogenesis, but by unknown mechanisms. Published data shows that DDX49 is involved in the progression of breast cancer, lung cancer and hepatocellular carcinoma. However, due to the lack of mechanistic insights, studies are needed to identify how catalytic activities of DDX49 are linked to it phenotypic effects, including how its dysregulation leads to cancer. We report biochemical activities of purified DDX49 in vitro, including nucleic acid binding and unwinding with substrate preference, an unexpected nuclease activity, and a hyperactive mutation of DDX49. We show that cells edited in DDX49 by CRISPR-Cas9 have strongly reduced cellular migration. This begins to elucidate DDX49 enzymatic activities contributing to its established or uncharacterised physiological roles and cancer cell progression.

POSTER NUMBER: 51

Comparison of miRNA expression in PGC cell cultures before and after freezing in different freezing media

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MATE university

MicroRNAs (miRNAs) belong to the small non-coding RNA group. They regulate many critical biological processes such as proliferation, cell cycle progression, differentiation, survival, and apoptosis in many cell types.
In our laboratory, the direct function of miRNAs in gene regulation during embryogenesis and in stem cells has been studied. We characterized the stem cell-specific miRNA clusters in mice, rabbits, and chickens. Recently we examined and described in chicken PGCs the miR-302/367 cluster, the most known miRNA cluster related to stemness and the miR-138 expression profile. MiR-138 is known as a tumour suppressor but also has an essential role in heat stress regulation.
In our newly established chicken PCG lines, we determined miR-302b-3p, miR-302b-5p, and miR-138 expression patterns. We found different expression profiles in these miRNAs before and after freezing and compared the male and female PGC lines.

POSTER NUMBER: 52

CCDC174 an uncharacterized factor at the interface of splicing and RNA degradation

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Messenger RNAs (mRNAs) are synthesized in the nucleus, where they either form export-competent mature mRNAs or are retained and degraded. A group of proteins called RNA binding proteins (RBP) coat mRNAs during their lifespan, forming mRNPs and participate in the regulation of RNA metabolism. While the role of many RBPs is understood, there are still many unknown factors. One such unknown factor is a protein named CCDC174.
Immunoprecipitation-mass spectrometry (IP-MS) of CCDC174 has shown a link to two other proteins – eIF4A3 and NRDE2. eIF4A3 is a core component of the exon junction complex (EJC), which marks mRNAs as spliced and acts as a platform, to which other proteins can bind and influence mRNA metabolism. NRDE2, is a binding partner of the nuclear exosome cofactor MTR4. In S. pombe, NRDE2 and CCDC174 were suggested to facilitate the degradation of unspliced mRNAs. Interestingly, while S. pombe contains the EJC core components, formation of the EJC was not observed.
Using reconstitution assays with purified proteins, we could demonstrate that CCDC174 and eIF4A3 interact directly and that CCDC174 supports the assembly of the EJC core, which is not a stable entity on its own in vitro. Moreover S. pombe CCDC174 does not bind eIF4A3, suggesting the network of interactions is different in human cells. We explored CCDC174's connection to NRDE2 as well. CCDC174 directly interacts with NRDE2 and the NRDE2/MTR4 complex, where CCDC174 increases the RNA-binding properties of the complex.
Furthermore, we have mapped the regions of CCDC174 required for eIF4A3 and NRDE2 binding, as well as discovered CCDC174 mutants that abrogate these interactions. We plan to introduce these mutants into cells and use IP-MS to analyze which partners of CCDC174 are lost compared to the wild type. This experiment will enable us to hypothesize and further test the function of CCDC174.

POSTER NUMBER: 53

Pro-survival role of LINC00116-encoded peptide Mitoregulin in Hodgkin lymphoma

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LINC00116 was first identified as a long non-coding RNA. However, several studies revealed that LINC00116 encode a 56-amino acid micropeptide, localized in the inner mitochondrial membrane, called Mitoregulin (MTLN). LINC00116 was shown to be upregulated in B-cell lymphoma compared to normal B-cells. However, the functions of both LINC00116 and MTLN in B-cell lymphoma are unknown. In this study, we focus on the role of MTLN in B-cell lymphoma. We showed that levels of both LINC00116 and Mitoregulin were significantly higher in Hodgkin lymphoma (HL) cells than in normal B-cells. We inhibited the expression of LINC00116 using two short hairpin RNAs (shRNAs) in lentiviral vectors. We showed that MTLN and LINC00116 levels decreased upon LINC00116 knockdown compared to negative controls. Since the vectors contained GFP, we examined the number of GFP+ transduced HL cells compared

to wild-type (WT). Within 21 days, the percentage of HL cells with inhibited LINC00116 decreased significantly compared to cells transduced with negative control vector. Therefore, LINC00116 silencing inhibited HL cell growth. Moreover, we demonstrated that this inhibition was not caused by apoptosis, since no significant difference in the apoptosis rate was observed for L540 HL cells with inhibited MTLN compared to negative controls within 20 days upon transduction.
In conclusion, we showed that MTLN is overexpressed in Hodgkin lymphoma and has a pro-survival role for HL cells. In further studies we aim to demonstrate possible differences in the function of LINC00116 and MTLN.
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POSTER NUMBER: 54

Structural and functional characterization of the human tRNA thiolation cascade

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tRNA modifications are essential for the proper and efficient functioning of protein translation. To date, more than 100 tRNA modifications have been identified. The exchange of oxygen with sulfur (thiolation) at the C2 position of wobble base uridines (U34) facilitates codon recognition, reading frame maintenance and accurate protein synthesis. In humans, the highly conserved CTU1-CTU2 complex carries out the final reaction step and modifies 4 tRNAs, namely tRNA Lys(UUU), tRNA Glu(UUC), tRNA Gln(UUG) and tRNA Arg(UCU). However, the exact mechanism of the CTU1-CTU2-mediated sulfur transfer reaction and the requirements for tRNA recognition as well as selectivity remain elusive. Thiolation of tRNAs is clinically relevant and it is crucial for the proper functioning of cells as well as organisms. The lack of uridine thiolation disrupts cellular protein homeostasis and leads to occurrence of various neurodegenerative disorders. Moreover, patient-derived mutations in the CTU2 subunit are associated with a rare congenital anomalies syndrome called DREAM-PL.
My PhD project aims to investigate and explain the mechanism of tRNA thiolation in humans and other eukaryotes at the molecular level. We heterologously produced the CTU1-CTU2 complexes from different organisms in insect cells and purified them to homogeneity. With the use of single particle cryogenic electron microscopy (cryo-EM) and complementary biochemical approaches, we now seek to understand the mechanism of the final step of the thiolation cascade. In detail, we aim to characterize the binding specificity of different tRNA substrates, to image different reaction intermediates at high-resolution and to identify the role of additional binding partners.

POSTER NUMBER: 55

Adar null mutant mice lacking the Adar1 RNA editing enzyme: effects of cell death mutations on survival and innate immune defect

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The deamination of the adenosine base to inosine in dsRNA is catalyzed by the ADAR RNA editing enzymes. In humans, mutations in ADAR1 that decrease RNA editing activity cause Aicardi Goutières Syndrome (AGS).
AdarΔ2-13 mutant mouse embryos die by embryonic day E12.5 with high interferon levels, widespread cell death, particularly death of hematopoietic cells in the fetal liver due to an aberrant innate immune response of the RLR Mda5 to unedited dsRNA. AdarΔ2-13 Mavs double mutant embryos lacking the Mavs adapter protein prevent signaling downstream of Mda5 and survive till birth but still die within a few days of birth.
We tested effects of a range of other mutations on birth, growth and lifespans of AdarΔ2-13 Mavs pups. Quadruple mutant newborn pups Adar Mavs Adarb1 (Adar2) Gria2R/R lacking all RNA editing activity survive 5 days longer than double mutants, possibly due to Gria2R/R. We crossed the Casp11129 (Caspase 11) mutant to AdarΔ2-13 Mavs to generate triple mutants. AdarΔ2-13 Mavs Casp11129 triple mutants did not extend pup survival indicating that Casp11 not critical to the early death of AdarΔ2-13 Mavs newborn pups. We also crossed in a Trp53 mutant removing P53 but Trp53 triple mutants were not obtained.
Aberrant activation of a second dsRNA sensor, Protein Kinase R (Pkr) can inhibit translation and drive stress granule formation, and cell death. To complete full rescue of pup survival, we generated AdarΔ2-13 Mavs Eif2ak2 (Pkr) triple mutants. Triple mutants do grow slowly and up to 80% survive long-term and can be used for breeding. We showed that the early death and severe gut defects in double mutant pups arising from death or aberrant differentiation of proliferating gut stem cells are rescued in AdarΔ2-13 Mavs Eif2ak2Ps1 triple mutant mice.

POSTER NUMBER: 56

Protective effects of inactive Adar1 protein and mutations reducing cell death in Adar mutant mouse pups

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The AdarE861A mutant encoding catalytically inactive Adar1 RNA editing enzyme is embryonic lethal but embryos survive 2 days longer than embryo with the AdarΔ2-13 null mutant. We made strains to compare rescues in AdarΔ2-13 null and AdarE861A catalytically inactive mutant backgrounds when either Mda5 or Mavs are also removed and aberrant interferon induction by unedited

endogenous dsRNA is prevented. As a result of these crosses, we generated many AdarE861A Mavs double homozygous mice, which are near normal size, suggesting a pretty complete rescue by removing Mavs. We also generated AdarE861A IfiH1 (Mda5) double mutant mice that appear fully rescued and reach breeding age. We are investigating the role of AdarE861A in intestinal apoptosis, which occurs in AdarΔ2-13 Mavs double homozygous pups but should not occur in the AdarE861A Mavs double mutant. Double mutant mice with AdarE861A and either Mavs or Ifih1 mutations appear well rescued.
However, when we use Adar E861A IfiH1 (Mda5) or Adar E861A Mavs double homozygous mutant female mice in crosses, all the progeny are small and die early. This is the case not only when the pups have the same Adar E861A IfiH1 (Mda5) or Adar E861A / Mavs double mutant genotype as the mother but even if they are heterozygous for AdarE861A. Therefore, there is a severe maternal defect in the Adar E861A IfiH1 or Adar E861A Mavs double homozygous others. Unpublished reports suggest that Adar knockout in endothelial cells of blood vessels recapitulates the severe blood-related defects seen in whole embryo Adar null mutants; therefore, the AdarE861A / E861A IfiH1- /- mothers might have placental insufficiency due to defects in placental blood vessel development. For this reason, we are examining placentae of at E18.5- E19.5 fetuses in all crosses to explain maternal effect on development of pups.

POSTER NUMBER: 57

Structural analyses of the human QTRT1/2 complex

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Transfer RNA (tRNA) plays a central role in decoding genetic information into proteins. Recent studies revealed that modifications in the tRNA anticodon regulate the speed and fidelity of ribosomal translation, supporting proper co-translational folding of the nascent polypeptide chain.
Queuosine (Q) is a modified nucleoside present at position 34 in four prokaryotic and eukaryotic tRNAs, namely tRNAAsp, tRNAAsn, tRNAHis and tRNATyr. However, only bacteria possess the full enzymatic pathway able to synthesize Q from GTP and to incorporate Q into tRNAs. Eukaryotes harbor only the enzyme that allows to conduct the last step of the modification pathway – the tRNA guanine transglycosylase (TGT) called queine tRNA-ribosyltransferase (QTRT). Thus, eukaryotes rely on exogenous supply of Q, but can incorporate Q into tRNA post-transcriptionally. Bacterial TGT acts as a homodimer, whereas human QTRT is a heterodimer, composed of the catalytic QTRT1 and non-catalytic QTRT2 subunits. Numerous studies showed the important role of Q for the regulation of protein synthesis and the activity of other tRNA modification enzymes. Moreover, queuosinated tRNAAsp and tRNATyr are further decorated with mannose and galactose, respectively.
Although many structural studies aimed to provide mechanistic information about the QTRT1 and QTRT2 enzymes, we still lack structural insights into the heterodimeric complex with a full-length substrate tRNA. My PhD project aims to provide a comprehensive understanding how four tRNAs are specifically recognized by QTRT1/2 complex and what is the exact mechanism of an enzymatic reaction. To achieve this goal, we have used single particle cryogenic electron microscopy (cryo-EM) to study the human complex and its reaction intermediates. We complement our structural work with various in-vitro biochemical assays.

POSTER NUMBER: 58

The role of SCAF1 in transcription regulation and co-transcriptional processing

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Transcription of protein coding genes is a dynamic process dependent on the RNAPII and regulated via its C-terminal domain (CTD). The CTD is dynamically phosphorylated at several sites during the transcription cycle to orchestrate recruitment of proteins involved in regulation of the transcription process and co-transcriptional processing. These RNAPII associated factors are crucial for correct mRNA biogenesis and proper gene expression; however, we still have an incomplete understanding of how RNAPII associated factors regulate co-transcriptional processing. To obtain a more comprehensive view of each of the multiple steps of mRNA biogenesis, it is crucial to identify different RNAPII interactors and their role throughout transcription.
One family of RNAPII associated factors is the Serine/Arginine (SR)-related CTD associated factors (SCAFs). Although all SCAF proteins contain RNAPII CTD-binding domains and interact with RNAPII, most of them remain uncharacterized. Recently, SCAF4 and SCAF8 were shown to share an essential role as mRNA anti-terminators, by preventing the early mRNA transcript cleavage. In this project, we focus on the role of the previously uncharacterized SCAF1 in transcription and co-transcriptional processing. Interestingly, SCAF1 binds the same RNAPII complexes as SCAF4 and SCAF8. With high-throughput experiments and sophisticated transcriptomic techniques, we show that SCAF1 binds the hyper-phosphorylated CTD of RNAPII and associates with elongation and 3' end processing factors. Moreover, loss of SCAF1 displays isoform switching, leading to expression of shorter mRNAs originating from early mRNA transcript cleavage events.

POSTER NUMBER: 59

6-Thioguanosine monophosphate prodrugs with the potential to overcome thiopurine resistance

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The clinical use of thiopurine drugs for the treatment of certain cancers and inflammatory diseases often results in side effects or resistance development. To overcome these issues, we synthesized different 6-Thioguanosine monophosphate (6sGMP) prodrugs

and tested them on various cell lines. Two of the six compounds show enhanced antiproliferative action against thiopurine-resistant cancer cells. Interestingly, our results indicate involvement of the purine salvage pathway enzyme HGPRT in this process, as its expression correlates with resistance to 6-thiopurine. By contrast, 6sGMP prodrugs do not need this enzyme for processing. We used the 6sG conversion method TUC-seq DUAL to show that the 6sGMP prodrugs are converted into their bioactive forms and incorporated into RNA. This method could be a new and straightforward way of measuring the success of thiopurine therapy via the incorporation of 6sG. Furthermore, our findings indicate that 6sGMP prodrugs can potentially be used as a new thiopurine treatment strategy to overcome resistance.

POSTER NUMBER: 60

Single-cell RNAi sensor system for assessment of increased RNAi efficiency

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RNAi is a biological response to long double-stranded RNA which results in sequence-specific mRNA degradation. Used as an ancestral antiviral innate immunity system but replaced by the interferon response during the vertebrate evolution, cells still conserve the machinery required for this primordial pathway.
Since it has been reported that the RNAi pathway can be restored to fulfill its original response, having an optimal system to measure RNAi efficiency is essential. Although a complex luciferase reporter system for endogenous RNAi has been developed and optimized, it still has certain limitations such as: i. single time-point measurement, ii. impossibility to determine heterogeneity of the reporter activity in individual cells, and iii. issues for high-throughput screening of miRNA activity. Therefore, we propose a fluorescence-based RNAi reporter system because of its greater flexibility in data collection.
Here we describe a fluorescence-based RNAi reporter system in the shape of a stable cell line which expresses knocked-in fluorescent proteins into highly expressed endogenous genes. This system allows us to monitor RNAi efficiency and covers the aforementioned limitations, while offering data collection flexibility including single-cell resolution and sampling the cell at multiple timepoints.

POSTER NUMBER: 61

High-throughput analysis of small drug-like molecules interacting with mRNA 3'UTRs

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Only a small portion of the human genome has been targeted by existing drugs as proteins. RNA thus becomes a promising target for small drug-like molecules because, similar to proteins, it can form pockets suitable for specific high-affinity binding with the above-mentioned compounds. The 3'-untranslated regions (3'UTRs) of mRNAs, which contain highly structured parts, are a good starting point for a small drug-like molecules screening. For the beginning, we selected some oncogenic and non-oncogenic mRNA with 3'UTRs of different length and structure. We designed a library of small drug-like molecules based on their ability to potentially bind different RNA structures. For a primary screening, we chose in-cell analysis with the dual luciferase assay among all current techniques because it is simple, rapid, and sensitive. In a secondary screening phase, we plan to further investigate positive hits by analysing RNA integrity in the samples with direct-cell one-step RT-qPCR. With this system in hands, we are now able to perform high-throughput screening of potential 3'UTR binders and identify candidates for further optimization.
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POSTER NUMBER: 62

Visualising the regulation of P-element transposon mRNA by the piRNA pathway in vivo

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Preventing the activation of transposable elements is a key layer of genomic and cellular protection, particularly in the crucial context of the germline. In the Drosophila germline, the silencing of transposons is mediated by piwi-interacting RNAs (piRNAs), which can act to repress targets through both chromatin-mediated transcriptional silencing and post-transcriptional slicing. However, piRNA-dependent repression of the P-element DNA transposon has been found to utilise alteration of P-element splicing to suppress transposition. This splicing regulation depends on several factors taking part in the canonical chromatin-mediated transcriptional regulation by piRNAs. Yet, previous work has shown no apparent change in P-element mRNA transcript accumulation in the presence/absence of piRNAs. To dissect this unusual regulatory mechanism at the tissue level, we have performed Single-Molecule Fluorescence In Situ Hybridisation (smFISH) using probes against P-element sense transcripts in the presence and absence of piRNAs. In the absence of piRNAs, we have found P-element transcripts in both the nucleus and the cytoplasm of germ cells. In contrast, in the presence of piRNAs, P-element transcripts exclusively accumulate within the nucleus of transcriptionally active germ cells. Interestingly, the drastic change observed for the cytoplasmic signal was not accompanied by equivalent changes in the nuclear signal or the total P-element mRNA accumulation, suggesting that piRNAs may regulate the export of P-element mRNAs to the cytoplasm. To investigate this further, we are coupling RNA visualisation techniques with genetic experiments to untangle the mechanisms responsible for post-transcriptional silencing and transcript nuclear export.

POSTER NUMBER: 63

RNA as a therapeutic target

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The majority of drugs currently available target proteins, but a large proportion of proteins, around 80%, are considered undruggable; therefore, selecting another suitable candidate is crucial for treating many systemic and cancer-related pathologies. Around 75 % of the human genome is transcribed into RNA, while only a small fraction (3%) of it translates to protein. Hence, RNA can be exploited as a potential therapeutic target. Specifically, the 3' untranslated regions (3'UTRs) of RNAs which play a crucial role in mRNA stability and gene expression regulation, have distinct secondary and tertiary structures that interact with proteins within cells. In this study, the goal is to identify small drug-like molecules that can target the 3'UTRs of several non-druggable oncogenes and non-oncogene addiction genes, such as MYC, KRAS, HSF1, CDK12, NRF2, etc.
A library of specialized heterocyclic compounds representing drug-like small molecules was screened against 120 nucleotide fragments of the MYC mRNA 3'UTR using a high-throughput fluorescence-based anisotropy assay (FA). Several fragments showed significant changes in fluorescence anisotropy when mixed with small molecules, indicating potential binding and conformational changes in the RNA structures. Selected RNA fragments were subsequently screened using Surface Plasmon Resonance (SPR), a highly sensitive biophysical technique where RNA is immobilized, and small molecules are flowed across the surface. Significant binders were identified, indicating the potential of these small molecules to interact with the RNA targets.
Our screening approach leverages the conformational changes in RNA fragments upon small molecule binding. Further characterization of these interactions will enable us to create a library of RNA motif-small molecule interaction pairs that can be utilized to target other mRNAs. These interaction pairs will aid in identifying lead compounds capable of modulating mRNAs and mRNA-protein complexes within cells. Disrupting RNA-protein interactions with small molecules can result in the downregulation of disease-related protein expression, with potential therapeutic implications.

POSTER NUMBER: 64

MicroRNA signatures in Parkinson's disease; functional role and clinical relevance

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Parkinson's disease (PD) is a neurological condition that affects movement. The disease is the second most common neurological condition, second only to Alzheimer's and the second most common movement disorder, with essential tremor being the most common. It is estimated that one per cent of the population over 65 will develop PD. Currently, the root cause remains elusive with links identified to specific genes or environmental factors or a combination of both. Analysis of MicroRNA (miRNA) may highlight the genetic mechanisms that contribute to pathogenesis of PD. Dysregulation of miRNA may allow for the early identification of PD, potentially allowing for medical intervention a significant amount of time before disease onset.
The SH-SY5Y cells was exposed to the neurotoxic compounds MPP+ and MG132 for 24 hours to cause 50 per cent cell death. The cells were extracted using TRIzol and purified using the mirvana kit. Following isolation and purification reverse transcriptase quantitative PCR analysis was conducted to observe the possible dysregulation of the genes, SNCA, ITPR1 and CACNA1C and the miRNA, miR-107, miR-153, miR-128a and miR34a when compared to wildtype cells with no neurotoxins.
Paired t-test was used to observe the delta-delta Ct for expression variation between wildtype cells and drug exposed cells.
The three genes, SNCA, ITPR1 and CACNA1C exposed to MPP+ were all significantly ($p = 0.05$) differentially expressed when compared to wildtype cells. SNCA exposed to MG132 was significantly different to wildtype. MiR-128a exposed to MG132 was significantly differentially expressed. Further analysis of miR-107, miR-153 and miR-34a will need to be conducted. Significant variation of expression can be observed in cells exposed to both drugs; however, further research needs to be conducted to observe if the remining genes and miRNA are significantly different to wildtype.

POSTER NUMBER: 65

Understanding the differences in ribosome binding capabilities of two dormancy factors Dap and Dap1b

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Zebrafish eggs present a translationally repressed environment, but translation is gradually activated during early embryonic development. Recently, our group unraveled that a network of conserved factors is bound to maternal ribosomes, stabilizing them and keeping them in a translationally inactive „dormant" state. Mass spectrometry and cryo-electron microscopy revealed Death-associated protein (Dap) and its paralog Dap1b as factors that insert via their C-termini into the polypeptide exit tunnel of dormant ribosomes in zebrafish and Xenopus eggs. However, the molecular mechanism of their ribosome binding and release from the ribosome remains elusive. Moreover, while Dap and Dap1b can both bind to egg ribosomes, Dap1b shows stronger binding than Dap both in vivo and in vitro. Which regions or domains in Dap/Dap1b explain the striking difference in binding is currently not understood.
To interrogate individual Dap/Dap1b domains for their differential ribosome binding behavior we generated mRNAs of multiple chimeric or truncated versions of Dap/Dap1b. We then translated these mRNAs in a flexible in vitro translation assay based on rabbit reticulocyte lysate and evaluated the produced proteins' binding efficiency to rabbit ribosomes.
Progressive substitution of C-terminal Dap amino acids for the corresponding Dap1b amino acids resulted in increased ribosome binding while the inverse decreased binding. Nevertheless, presence of both the N- and C-termini was indispensable for association

to the ribosome. Even more so, synthesized N-/C- termini of Dap/Dap1b efficiently competed with full length dap/dap1b for ribosome binding, indicating an important role for either terminus in binding the ribosome. As Dap and Dap1b display substantially different pI-values, we hypothesized that overall protein charge plays a role. Accordingly, we uncovered a correlation between binding affinity to ribosomes and protein pI-value. Prospectively it will be interesting to use our in vitro findings to understand the molecular processes that govern Dap/Dap1b ribosome association and release in vivo.

POSTER NUMBER: 66

Reactivation of RNA interference pathway in mammals

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

POSTER NUMBER: 67

Transposable elements and the forces driving genome evolution

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Transposable elements (TEs) are found in nearly all genomes and are often major constituents, making up almost half of the genomic sequence in humans and around 20-30% in flies. These mobile genetic elements can proliferate and jump from one locus to another, thereby increasing in copy number, which can have substantial impacts on their hosts' genomes. While these can be sometimes beneficial for the host, e.g. by providing raw material for the evolution of gene regulation, they also pose a threat to genome integrity and function. Therefore, host defence mechanisms, such as the metazoan germline specific PIWI/piRNA system, have evolved to keep such selfish DNA modules in check. The major piRNA-producing loci, called piRNA clusters, which mostly consist of TE insertions on the sequence level, are considered to be key regulators of active TEs throughout the rest of the genome. However, we have recently challenged this model in the fly germline by showing that they are largely dispensable for TE repression, which raises the question of why and how piRNA clusters emerge. Since piRNA clusters have a relatively rapid turnover rate during evolution and tend to emerge in breakage-prone genomic regions, we hypothesise that piRNA clusters in flies are a product of local evolutionary pressure relaxation and toleration of TE accumulation at sites of reduced recombination rates, such as at inversion breakpoints and in proximity to centromeres. To elucidate the relationship of piRNA clusters and TEs within genome evolution more broadly, we are integrating germline small RNA sequencing data with recent nanopore-sequenced genome assemblies that are additionally scaffolded to chromosome-level quality with HiC data from 32 species of the Drosophila genus. The comparative analysis of piRNA loci and inversion breakpoints is complemented by tracking of 3D genome structure evolution, adding yet another perspective into the evolutionary dynamics of TE-host interaction.

POSTER NUMBER: 68

Staufen dimerization and binding to RNA

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Human Staufen1 (hStau1) is a protein mostly known for its involvement in mRNA transport and degradation. Staufen binds to stem-loop structures in the 3'UTR of its mRNA targets. Once this event occurs, hStau1 homodimerizes and induces a UPF1-dependent mRNA degradation known as Staufen-Mediated mRNA Decay (SMD). hStau1 dimerization and binding to RNA are therefore considered key events in mRNA degradation, gene expression, cancer development, neurodegenerative diseases and many other biological events of tremendous importance. This protein contains the following domains: N-double strand RNA Binding Domain 2 (dsRBD2), dsRBD3, dsRBD4, Tubulin Binding Domain (TBD), Staufen-Swapping Motif (SSM) and dsRBD5-C. In order to understand the Staufen dimerization and binding to RNA, we analyzed hStau1 and its derivatives by Analytical UltraCentrifugation (AUC), Small Angle X-ray Scattering (SAXS) and Fluorescence Anisotropy (FA). SAXS data suggests that hStau1 is characterized by several elongated tertiary structures. Our AUC data shows that SSM deletion dramatically reduced Staufen dimerization. However, the progressive deletion of dsRBD2, dsRBD3, dsRBD4 and TBD did not significantly affect this phenomenon. Moreover, we also noticed that Mg2+ cations stimulated Staufen dimerization. According to our FA data, hStau1, hStau1ΔSSM, RBD3-End and RBD3-EndΔSSM did not display significant differences in RNA binding affinity. However, dsRBD3 deletion caused strong reduction in Staufen affinity to RNA. In conclusion, hStau1 and its derivatives are characterized by several tertiary structures, this dynamism may be necessary to interact with multiple ligands and play different roles. Our data suggests that dsRBD2, dsRBD3, dsRBD4 and TBD are not involved in Staufen dimerization. On the other hand, dsRBD2 and SSM do not significantly affect Staufen-RNA binding. Overall, our findings provide insights into the structural and functional features of hStau1 and its derivatives, which may have important implications for understanding the protein's role in various biological processes.

POSTER NUMBER: 69

SNOMATCHER: IDENTIFICATION OF CANDIDATE GUIDE SNORNAS FOR NEWLY-DISCOVERED 2'-O-METHYLATIONS

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The most abundant post-transcriptional modifications of ribosomal RNA (rRNA) are 2'-O-methylations (2'-O-Me). They are likely major contributors to ribosome heterogeneity, which has been implicated in translation regulation and human diseases. Methylations are primarily catalyzed by methyltransferase fibrillarin, guided to the rRNA target by C/D box small nucleolar RNAs (snoRNAs), which are short RNAs processed from lariat introns of other genes.

The entire landscape of 2'-O-methylations in a biological sample can be obtained using RiboMethSeq. We exploited this technique to observe differences in methylation patterns between total rRNA and rRNA incorporated into mature ribosomes in mouse brain. Results revealed a number of still-unknown 2'-O-methylations, which do not have assigned guide snoRNAs. In fact, databases provide incomplete information and cover only human snoRNAs. Furthermore, available snoRNA-prediction tools are not suited for the detection of guide snoRNAs starting from newly-discovered 2'-O-methylations.

For these reasons, we developed SnoMatcheR, an R shiny web app capable of matching novel methylation sites to putative guide snoRNAs at genome-wide level and in an unbiased manner. SnoMatcheR identifies snoRNA-like stretches in target sequences from transcriptomes or genomes, based on snoRNA structure and base pairing properties with 2'-O-Me sites. SnoMatcheR detects antisense region and flanking C and D boxes of putative snoRNAs, from which a probability score of being a bona fide snoRNA is generated. We validated our tool on a set of 108 human 2'-O-methylations with assigned snoRNAs retrieved from the SnoDB 2.0 database, and it identified guide snoRNAs for 99% of the sites.

Therefore, SnoMatcher performs genome-wide searches to identify putative guide snoRNAs for any set of newly-identified methylation sites in human and mouse, as well as in unannotated species. Taken together, our results show that SnoMatcheR is an essential prediction tool to complete the snoRNAome and consequently explore novel translation regulation mechanisms in physiological and disease conditions.

POSTER NUMBER: 70

Interplay between the cytoplasmic m6A readers ECT2/3/4 , ECT5 and ECT7 shapes plant architecture

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N6-methyladenosine (m6A) in mRNA is important for eukaryotic gene regulation and is indispensable for the development of plants and vertebrates. The best understood mechanism of m6A-dependent gene control involves association of m6A-containing mRNA with cytoplasmic RNA-binding proteins specialized for m6A-recognition via a YTH521-B homology (YTH) domain, the so-called YTHDF proteins. The flowering plant Arabidopsis encodes eleven YTHDF proteins, named EVOLUTIONARILY CONSERVED C-TERMINAL REGION (ECT) 1-11. Simultaneous knockout of ECT2, ECT3 and ECT4 causes developmental delay, dwarfism and morphological defects reminiscent of those observed in plants partially defective in m6A deposition, but weaker with regard to organ size of adult plants. Thus, it is a pertinent question whether additional YTHDF proteins mediate m6A-dependent regulation of plant development. Here, we show that while ECT2 and ECT3 associate in wild type plants, and that ECT2 interacts with ECT5 and ECT7 in the absence of ECT3. Such association suggests a cooperative regulation of common targets and, indeed, additional mutation of ECT5 or ECT7 exacerbates developmental defects of ect2/3 or ect2/3/4 mutants. In particular, ECT5 plays important roles in defining plant stature, explaining much of the difference observed between ect2/3/4 mutants and those partially defective in m6A deposition. Our results establish that YTHDF proteins mediate the most readily detectable function of m6A in plant post-embryonic development, and provide new insight into the mechanistic basis for plant YTHDF action.

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