ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DEPARTMENT
OF MEDICAL
AND SURGICAL SCIENCES

RiboMod2025
snoRNA and rRNA modification

RiboMod2025

Tools and Methods for the Annotation and Study of snoRNA-Mediated Ribosomal RNA Modifications

Abstract book

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• Introduction •

Dear colleagues,

We are excited to welcome you to the RiboMod2025 Workshop!

In recent years, the field of snoRNA and snoRNA-guided ribosomal RNA modifications has experienced a significant surge in innovative detection and study methods. However, this rapid expansion has introduced challenges such as inconsistencies in dataset comparisons, snoRNA naming conventions, and the identification of modification sites. RiboMod2025 is designed to address these issues by standardizing and streamlining research practices across the field.

RiboMod2025 isn't just another conference; it's a targeted workshop focused on establishing standardized practices for naming and studying snoRNA and snoRNA-guided ribosomal RNA modifications. This event bring together leading experts from all corners of the field, including method developers, researchers utilizing diverse model systems, database administrators, science writers, and editors. The goal is to foster a collaborative environment that encourages the exchange of ideas and sets a unified direction for future research. The two-day meeting will feature morning sessions of provocative talks highlighting specific challenges within the field. These presentations will set the stage for intensive small-group discussions among experts who will debate and formulate recommendations. These sessions will be documented by professional science writers and moderated by skilled facilitators to ensure productive and focused discussions. The meeting will also feature a poster presentation session and oral presentations selected from submitted abstracts. Attendees will also have the opportunity to engage in the small group discussions.

Each discussion group's recommendations will be meticulously curated and reviewed in a comprehensive session involving all participants. This collective review aims to finalize and agree upon the **guidelines** that will form the basis of a published recommendation letter, endorsed by all attendees. This document will serve as a foundational reference for future research and publications in the field. The documents will be published as service for the entire community and will be submitted as guide for all publishers in the field.

Thank you to participating in RiboMod2025 and contribute to shaping the future of snoRNA and snoRNA-guided ribosomal RNA modification research. This workshop offers a unique opportunity to influence standard-setting decisions that will impact how research is conducted and communicated in the years to come. The workshop also allows the participants to engage in a highly inclusive and dynamic environment and to make a lasting impact on this crucial scientific domain.

The Scientific Organizing Committee

Sherif Abou Elela (Sherbrooke, Canada)
Katherine Bohnsack (Goettingen, Germany)
Homa Ghalei (Atlanta, USA)
Denis L.J. Lafontaine (Brussels, Belgium)
Virginie Marcel (Lyon, France)
Lorenzo Montanaro (Bologna, Italy)
Davide Ruggero (San Francisco, USA)
Michelle Scott (Sherbrooke, Canada)

• Program at a glance •

• June 12th, 2025 • Small nucleolar RNAs •

8.30 AM – 9 AM	Registration and Welcome Coffee
9 AM – 9.20 AM	Welcome and Introduction
9.20 AM – 10.20 AM	**SESSION 1** Methods for Detecting and Characterizing snoRNA
10.20 AM – 10.50 AM	Coffee Break
10.50 AM – 12.00 PM	**SESSION 2** Databases, Classification, and Validation of snoRNA
12.00 PM – 12.50 PM	**SESSION 3** Methods for Functional Analysis of snoRNAs and rRNA modifications
12.50 PM – 2.00 PM	Lunch Break
2.00 PM – 4.00 PM	**TOPIC DISCUSSIONS**
4.00 PM – 4.30 PM	Coffee Break
4.00 PM – 5.30 PM	**POSTER SESSION**
7.45 PM	Guided Tour / Walk
8.30 PM	Dinner / Party

• June 13th, 2025 • Ribosomal RNAs •

8.30 AM – 9 AM	Registration and Welcome Coffee
9 AM – 10.10 AM	**SESSION 4** Methods for Detecting and Quantifying Ribosomal RNA Modifications
10.10 AM – 10.40 AM	Coffee Break
10.40 AM – 11.50 AM	**SESSION 5** Methods for Assaying the Function of Ribosomal RNA Modifications
11.50 AM – 12.50 PM	**SESSION 6** Methods and Approaches for Identifying RNA Binding by snoRNAs
12.50 PM – 2.00 PM	Lunch Break
2.00 PM – 4.00 PM	**TOPIC DISCUSSIONS**
4.00 PM – 5.00 PM	Coffee Break
5.00 PM – 6.00 PM	**GENERAL ROUND TABLE** Presentation of Recommendations and Reaching Consensus
6.00 PM – 6.15 PM	**CEREMONY AWARD**
6.15 PM – 6.30 PM	Concluding Remarks

• Abstracts Oral Presentations •

S1-O2

Toward Reliable snoRNA Quantification: Benchmarking Reverse Transcriptases and Methodological Controls

Sherif Abou Elela

Département de microbiologie et d'infectiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Canada

Small nucleolar RNAs (snoRNAs) are essential regulators of ribosomal RNA modification, yet their short length and stable secondary structures present major technical hurdles for accurate sequencing and quantification. To address these challenges, we systematically evaluated the impact of key methodological variables-from RNA extraction to data analysis-on snoRNA detection. While multiple factors, such as RNA extraction and computational analysis pipelines, can influence results, the choice of reverse transcriptase (RT) proved the most significant. Using synthetic mouse snoRNA spike-ins as normalization controls, we compared the performance of high-processivity RTs (including TGIRT, MarathonRT, and Induro RT) with that of conventional enzymes (M-MuLV RT and related variants). High-processivity RTs consistently outperformed standard enzymes, yielding greater snoRNA recovery and more uniform coverage across structured regions. The inclusion of synthetic spike-ins further enhanced reproducibility, substantially reducing technical variation and enabling reliable cross-study comparisons. These findings build on prior studies demonstrating the advantages of thermostable RTs for structured RNA detection and extend the evaluation to newer, high-performance enzymes. Our results highlight the importance of adopting standardized workflows that prioritize the use of high-fidelity RTs, integrate spike-in controls, and transparently report all library preparation details. Such methodological rigor is crucial for advancing research on snoRNAs in disease contexts and for establishing robust, reproducible protocols that can be widely adopted by the research community.

S1-O3

snoRNAs or snoRTs: how can we accurately quantify them?

Giulia Venturi, Guglielmo Rambaldelli, Sidra Asghar, Federico Zacchini, Lorenzo Montanaro

University of Bologna, Italy

Small nucleolar RNAs (snoRNAs) are non-coding RNAs whose genes are hosted within the introns of both protein-coding and non-coding genes. Recently, NGS data obtained from cancer samples derived from various tissues have revealed alterations in snoRNA expression compared to non-cancerous tissue, suggesting a potential role in cancer development. Moreover, many snoRNAs have been detected as circulating RNAs in the plasma of cancer patients, supporting their potential use as cancer biomarkers in liquid biopsy. However, a significant fraction of snoRNA sequences is found as retained introns of specific mRNA isoforms expressed by their host genes—referred to as snoRNA-retaining transcripts (snoRTs). By reanalyzing publicly available Oxford Nanopore Technology data, we found that snoRTs are widely represented in both human normal tissues and cancer-derived cell lines, appearing in their full-length form and, frequently, as truncated variants. We characterized the truncation sites, which occur at or very near the retained snoRNA sequence at the 5′ end. Interestingly, for some transcripts, this shorter variant is the only form detected. Additionally, some snoRTs are localized within the cytoplasmic fraction of the cell. For selected snoRNAs, we developed a droplet digital PCR assay to quantitatively assess the abundance of snoRT sequences in comparison to canonical snoRNA sequences. Our findings indicate that a variable but consistent proportion of snoRNA sequences in cells, tissues, and liquid biopsy samples are present as snoRTs. This highlights the need to carefully consider snoRTs when evaluating snoRNAs as biomarkers. Given that short-read and gene-based transcriptomic analyses often overlook these transcripts—sometimes leading to a misrepresentation of snoRNA sequences—greater attention must be paid to the selection of protocols used for snoRNA quantification. For instance, implementing RNA size-selection steps designed to exclude longer transcripts could effectively remove snoRTs, thereby improving the accuracy of snoRNA quantification.

S1-O4

Expanding the Capabilities of snoRNA Detection and Modification Profiling with NERD-seq

Athanasios Zovoilis

University of Manitoba, Canada

The detection and characterization of snoRNAs and their associated RNA modifications remain limited by methodological biases in transcriptome profiling. A persistent challenge is the underrepresentation of short non-polyadenylated, structured RNAs—such as snoRNAs—by standard sequencing protocols, especially Nanopore direct RNA sequencing. This exclusion narrows the scope of detectable snoRNAs and associated RNA modifications.

We address this limitation with NERD-seq (Non-coding Enriched RNA Direct sequencing), a new method developed in our lab that enables simultaneous profiling of both polyadenylated and non-polyadenylated RNAs without the need for transcript-specific adaptors. NERD-seq significantly increases coverage of structured ncRNAs—including snoRNAs—while retaining information-rich basecalling signals indicative of RNA modifications. Using mouse and human brain tissue, we demonstrate that NERD-seq recovers basecall and mismatch patterns associated with RNA modifications, while also capturing SINE- and LINE-derived transcripts missed by conventional protocols.

This work raises a number of questions for the field: What are we missing when we rely on standard poly(A)-based RNA sequencing? How can we build consensus around more inclusive, modification-aware transcriptomic strategies? As the field moves toward standardization in snoRNA and rRNA modification research, we propose that methods like NERD-seq offer a viable path forward for incorporating a broader range of non-coding RNAs into comparative studies and nomenclature frameworks.

S2-O5

Nomenclature issues from the perspective of RNAcentral and Rfam

Blake Sweeney, Andrew Green, Carlos Ribas, Nancy Ontiveros, Alex Bateman

EMBL-EBI, UK

RNAcentral (http://rnacentral.org) and Rfam (http://rfam.org) are two highly used resources in non-coding RNA science. RNAcentral is a database of non-coding RNA sequences, while Rfam focuses on ncRNA families. One common theme across both resources is to provide a representation of the scientific community's knowledge about ncRNAs. This task involves finding, annotating, and naming many ncRNAs, including snoRNAs.

Rfam captures knowledge through collaborations and reviewing literature. We collect alignments, names and other metadata and aim to include that as accurately as possible into Rfam. RNAcentral captures scientific knowledge in two ways. First, we integrate data from over 50 different databases into a single resource. This involves collating a wide range of identifiers, selecting representative descriptions, and in some cases normalisation. Secondly, we use our text mining and summarisation tools, LitScan and LitSumm, to produce summaries of literature. We use our comprehensive collection of gene and transcript identifiers and search literature for all mentions and then produce summaries. These activities require clear, consistently used, and unambiguous identifiers.

In this talk, we will use our experience to describe nomenclature issues and solutions observed in other ncRNA communities. We will describe nomenclature systems used by different ncRNA fields and databases and then describe issues with them. In particular, we will draw on recent work with miRNAs in Rfam and RNAcentral. We will highlight issues with cross organism identifiers, ambiguity, and issues arising from different standards between databases. We will focus on these issues in the context of running databases and our more recent work in text mining literature.

Finally, we will discuss the needs of databases and what the community can do to help us use and, when possible, enforce selected nomenclature. We aim to help the snoRNA community avoid some existing pitfalls that have been observed.

S2-O6

Annotating snoRNAs in GENCODE

Zoe Hollis, Jane Loveland, Jonathan Mudge,
Jose Gonzalez, Adam Frankish

EMBL-EBI, UK

The GENCODE consortium provides comprehensive reference gene annotation for the human and mouse genomes, including protein-coding genes, pseudogenes, long non-coding RNAs, and small RNAs. Accurate gene annotation is fundamental for genome biology and clinical genomics; incorrect or incomplete annotation impacts downstream analysis and introduces potentially significant errors. GENCODE genes and transcripts have been made predominantly by expert human annotation, with manually supervised automated methods playing an increasingly significant role. The annotation of small RNAs has differed in that it has been imported directly from expert databases such as miRBase and Rfam with only very minimal manual intervention. This has created challenges for the integration of all sRNA annotations into GENCODE, many of which specifically affect snoRNAs. For example, we have identified the following challenges:

1) Duplicated entries for some snoRNAs with different IDs at the same location
2) Identifying the correct start and ends of the snoRNAs, currently no standardisation between databases
3) Categorising and displaying the snoRNA types e.g., C/D box and H/ACA box in the genome browsers
4) Validation of functional snoRNAs
5) Annotation of all stages of the RNA biogenesis e.g., host genes, any intermediates, and the mature RNA and integrating this with other GENCODE annotation
6) Application to the pangenome

We aim to ensure that any annotation or amendments to annotation benefit the user community. As such, we are very keen to discuss annotation challenges and integrate expert contributions to overcome them, ensuring that our GENCODE catalog will be better aligned with the latest knowledge on snoRNA biology.

S2-O7

Identification of rRNA modifications and their corresponding snoRNAs in Drosophila melanogaster

Jean-Yves Roignant

University of Lausanne, Switzerland

During their maturation, ribosomal RNAs (rRNAs) are decorated by hundreds of chemical modifications that participate in proper folding of rRNA secondary structures and therefore in ribosomal function. Pseudouridine (Psi) and methylation of the 2′-hydroxyl ribose moiety (Nm) are the most abundant modifications of rRNAs. The majority of these modifications in eukaryotes are placed by conserved protein complexes guided by small nucleolar RNAs (snoRNAs). These modifications impact interactions between rRNAs, tRNAs and mRNAs, and some are known to fine tune translation rates and efficiency. In this presentation, I will discuss the work we have made in Drosophila melanogaster to build comprehensive maps of Psi and Nm sites on rRNAs using several orthogonal approaches. I will also describe our efforts to identify their corresponding snoRNAs by whole-transcriptome sequencing and how we revisited their nomenclature to better reflect their function.

S2-O8

Comprehensive Annotation and Expression Dynamics of the Zebrafish snoRNAome

Renáta Hamar and Dr. Máté Varga

Eötvös Loránd University, Budapest, Hungary

Small nucleolar RNAs (snoRNAs) are a conserved class of non-coding RNAs primarily involved in guiding chemical modifications of ribosomal RNAs, with emerging roles in various cellular processes and disease. However, our understanding of their repertoire and regulation remains incomplete, particularly in non-mammalian model organisms.

In this work, we present the first comprehensive annotation of the snoRNAome in zebrafish (Danio rerio), leveraging size-fractionated RNA sequencing data and a customized computational pipeline. Using conservative predictive methods we identified 67 previously unannotated snoRNAs, significantly expanding the known landscape of snoRNAs in zebrafish. Our results reveal dynamic snoRNA expression patterns during early development and distinct tissue-specific profiles in adult zebrafish, indicating a level of regulatory complexity previously underappreciated. To facilitate further research, we have developed snoDanio, a user-friendly online platform that enables interactive analysis of snoRNA expression in zebrafish.

We aim to contribute to ongoing efforts to develop reproducible and standardized resources for the RNA modification community. By providing both a well-annotated dataset and an accessible analysis framework, our work supports future research into snoRNA function and its relevance in development and disease.

S2-O9

Nomenclature and annotations to facilitate the study of snoRNAs eukaryote-wide

Michelle S Scott

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Small nucleolar RNAs (snoRNAs) are an ancient class of noncoding RNA present in all eukaryotes. Best characterized for their role in the biogenesis of ribosomes through their capacity to base pair with ribosomal RNA, snoRNAs are now known as versatile regulators modulating gene expression and multiple other cellular processes. The characterization of these varied roles has revealed diversity in snoRNA characteristics including snoRNAs targeting other types of RNAs, snoRNA that might not always interact with the same sets of proteins, snoRNAs that can localize to non-nucleolar and even non-nuclear cellular compartments to carry out their functions and that have non-standard sequence features. In addition, while many snoRNAs hark back to the last eukaryotic common ancestor, snoRNA genes are mobile, can move around in genomes, and are good substrates for gene duplication, with evidence of some very young members playing important functional roles in specific eukaryotic clades or even smaller groups of organisms.

Studying snoRNAs across organisms has the potential to increase our understanding of their evolution and improve their function annotation. However, many organisms are very poorly annotated for snoRNAs, and their current annotations are not readily comparable across organisms. This presentation will discuss

- nomenclature providing basic snoRNA characteristics such as snoRNA type (e.g. C/D vs H/ACA) that could facilitate cross-species comparison,

- the types of evidence that could be required to report a new snoRNA gene (the pros and cons of considering different features will be discussed: length, presence of motifs, structure, cellular localization, protein dependencies and binding partners, evidence of expression),

- the many snoRNA copies that exist in some eukaryotic genomes, most of which have never been detected as expressed and the idea of snoRNA pseudogenes.

Defining the most important recommendations to increase the quality of our snoRNA annotations can considerably facilitate future research on snoRNAs.

S3-O10

Assigning functions to ribosomal 2′-O-methylations

Anders Lund (on the behalf of the lab)

University of Copenhagen, Denmark

2′-O-methylation (2Ome) is the most abundant class of modifications found on ribosomal RNA. Using Ribo-meth-seq, we have mapped the pattern of 2Ome across more than 250 different cell and tissue types, including cancers, stem cell differentiation models, and neurons. We find that about 75% of the approximately 114 2Ome sites are highly methylated in the ribosome population across all samples analyzed, whereas the remaining around 25% displayed sub-stoichiometric methylation with levels differing between cell and tissue types and between normal and disease tissues. Using classical gain/loss-of-function studies, we demonstrated the roles of a few 2Ome sites.

In this presentation, data will be presented on 1) a CRISPR screening approach to identify individual snoRNAs and their cognate 2Ome sites affecting particular cellular functions, and 2) a single rRNA sequencing approach to determine which 2Ome appear on the same rRNA molecule in a first attempt to classify ribosome subtypes.

S3-O11

An Unexpectedly Large Repertoire of Box C/D Small RNAs Revealed in Mouse Tissues

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Box C/D small RNAs are a highly conserved and evolutionarily ancient class of antisense small non-coding RNAs found in both eukaryotes and archaea, and are thought to have originated from IS110-like transposon components. The vast majority function as guide RNAs, directing site-specific 2′-O-ribose methylation and, to a lesser extent, N4-acetylcytidine modifications. In mammals, the repertoire of box C/D RNAs has steadily expanded in recent years, with several hundred genes identified in the human genome to date. To better understand the complexity of this RNA family in understudied tissues, we performed high-throughput sequencing of Fibrillarin-associated RNAs in adult mouse brain, as well as in developing (e15.5) brain, placenta and whole trunk. Surprisingly, our analysis uncovered over 10,000 small RNAs exhibiting hallmark features of box C/D RNAs, including: (i) a characteristic kink-turn motif formed by conserved C- and D-boxes; (ii) stereotypical positioning of the 5′ and 3′ termini at 4-5 and 2-5 nucleotides from the C- and D-boxes, respectively, independent of terminal stem length; and (iii) enrichment of a uridine residue upstream of the D-box. Unlike canonical box C/D small nucleolar RNAs involved in rRNA modification, these newly identified candidates are typically lowly expressed, poorly conserved and unusually short, with over 60% measuring fewer than 50 nucleotides. These findings reveal a previously unrecognized and extensive layer of box C/D-like RNAs expressed in vivo, raising intriguing questions about their potential biological functions and evolutionary origins.

S3-O12

Decoding specialized ribosomes: challenges in functional validation of rRNA modifications

Mie Monti*, Ivan Milenkovic*, Martin Gigerey, Sonia Cruciani, Laia Llovera, Laura Battlé and Eva Maria Novoa

Centre for Genomic Regulation (CRG), Barcelona, Spain

A central challenge in studying specialized ribosomes is determining which ribosomal RNA (rRNA) modifications are biologically meaningful. Systematic profiling approaches, such as direct RNA nanopore sequencing (DRS), provide a powerful and unbiased means of identifying dynamic modification sites—those that vary across development or tissue types—and may thus represent regulatory, functional candidates. However, shortlisting these sites is only the first step. The field still lacks standardized, reliable strategies to functionally validate their roles.

We used DRS to map rRNA modifications across murine tissues and developmental stages. Among the dynamic candidates, 2′-O-methylation at position 18S:355 (18S:355Um) was notably enriched in terminally differentiated tissues. This dynamic behaviour was recapitulated in vitro by differentiating mouse embryonic stem cells (mESCs) into neural progenitor cells (NPCs) and neurons, where 18S:355Um levels inversely correlated with proliferation. SNORD90 was identified as the guiding snoRNA, and we generated CRISPR knockout (KO) mESC lines for functional analysis. SNORD90 KO mESC cells could be differentiated both to NPCs and neurons without apparent morphological differences compared to the parental strain. However, to our surprise, SNORD90 KO NPCs showed widespread transcriptional dysregulation compared to the parental strain, raising concerns that snoRNA KOs may elicit confounding phenotypes unrelated to the loss of a single rRNA modification.

To begin decoupling these effects, we are testing a non-targeting SNORD90 mutant in which the guide sequence is scrambled: preserving the C/D box structure while preventing site-specific rRNA binding. Additionally, the link between 18S:355Um and proliferation prompted us to investigate rRNA modification dynamics across the cell cycle. Our current work highlights both the promise and pitfalls of current functional assays and underscores the need for novel and unbiased methods to functionally characterize the role of specialized ribosomes in diverse biological systems.

S4-O13

First generation sequencing-based methods for RNA-guided rRNA modifications

Henrik Nielsen

University of Copenhagen, Denmark

Ribosomal RNA modifications are generally conserved in evolution. Yet, ablation of individual modifications rarely has a clear phenotype. Furthermore, rRNA modifications are relevant both in the context of ribosome biogenesis (role of the modifier in the assembly process) and the mature ribosome (role of the modification in translation). Thus, the relative contributions of modifier and modification must be considered for each site. This is particularly evident for the RNA-guided modifications (2´-OMe, ¬) that show considerable evolutionary dynamics and functional diversification.

Mapping of rRNA modifications in the main model organisms has largely reached consensus, although the modifier is often assigned based on established rules rather than experimental evidence. The main question is criteria for acceptance of de novo mapping given that both bioinformatics and experimental approaches have significant FP and FN rates. As a complement to criteria, a vocabulary describing the confidence of sites in new organisms should be developed.

Once the core mapping of sites is in place, subsequent experimentation will depend on methods for modification stoichiometry. Most methods are ensemble methods and some provide robust measurements. As the field moves towards single-molecule based methods, new questions appear, e.g. what qualifies as absolute quantification, how to compare relative changes, and how to describe pattern changes. Changes are often validated by single-site methods involving PCR-amplification and it is critical to define best practise for such methods.

The classical approach to nucleotide numbering is to facilitate comparison between species (as with group I intron insertion sites in rRNA), or molecular variants (tRNA). The pragmatic solution for rRNA was to use an old, not strictly correct, human reference sequence to ensure transparency with previous literature. Given that the modifications are overwhelmingly found in the conserved core, a structure-based nomenclature would be ideal, perhaps taking advantage of modern approaches to describe positional information.

S4-O14

LC-MS based quantification of modified ribonucleosides: an effort towards global harmonization of protocols and standards

Mark Helm

[1] *Institute for Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany*
[2] *The Human RNome Project Consortium*

The combination of liquid chromatography and mass spectroscopy (LC-MS) is ideally suited to quantify modified ribonucleoside, e.g. as obtained after enzymatic digestion of total RNA or isolated RNA species. Thus quantification of RNA modifications in ribosomal RNA (rRNA) is straightforward, while quantification in lower abundant species requires prior purification, which imposes serious limitations. Yet, even for abundant species like rRNA, the dependence of experimental results on various technical parameters poses problems in inter-laboratory comparability. Here we report a multi-laboratory effort to optimize and standardize the workflow for LC-MS/MS RNA modification analysis. We compared protocols for sample shipment, RNA digestion, LC-MS/MS analysis, and data processing among three laboratories working with the same total RNA samples. We were able to detect and quantify 17 modifications in total RNA consistently across protocols and operators, with another 7 that proved to be sensitive to experimental conditions, reagent contamination, and ribonucleoside instability, leading to poor precision among laboratories. These findings establish a robust and readily adoptable RNA modification analytical platform that enables reliable comparisons across laboratories studying the epitranscriptome.

S4-O15

Decoding the rRNA modification landscape using native RNA nanopore sequencing

Eva Novoa

Centre for Genomic Regulation (CRG), Barcelona, Spain

Ribosomes are now surveyed as dynamic entities that can be heterogeneous in their composition. This heterogeneity can arise from the use of ribosomal protein paralogs, distinct rRNA variants or differential rRNA modifications, among others.

While the rRNA modification landscape has been previously characterized for some species, most studies and rRNA databases do not take into account the tissue and/or cell type of origin in their annotations. While the different types of rRNA modifications are likely interconnected, detailed maps of all rRNA modification patterns are lacking.

In this context, direct RNA nanopore sequencing (DRS) has emerged as a promising technology that can overcome these limitations, as it is in principle capable of mapping all RNA modifications simultaneously, in a quantitative manner, and in full-length native RNA molecules. Notably. previous works have already shown that rRNA modifications can be identified using DRS.
Here, I will first present an overview of how we can use DRS to study the rRNA epitranscriptomic landscape. I will then illustrate how some of these methods can be used to identify different rRNA modification patterns across tissues, cell types, developmental stages and cancer types, which we validate using orthogonal methods. I will finally discuss how to use DRS to explore the rRNA modification landscape in individual RNA molecules, and what are the future steps moving forward.

S4-O16

Sci-ModoM: roadmap to facilitate access to and sharing of high-throughput transcriptome-wide RNA modification data, to harmonize data exchange formats and interoperability standards

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We recently presented Sci-ModoM[1], the first next-generation RNome database providing a one-stop source for RNA modifications originating from state-of-the-art high-resolution detection methods. With Sci-ModoM, we aim to address critical challenges facing the epitranscriptomics community such as the need for common standards and guidelines for reporting RNA modifications. Sci-ModoM consolidates and integrates RNA modifications reported across different studies into a single, easily accessible platform. In particular, it provides quantitative measurements per site and per dataset that allow to assess the confidence level of the reported modifications across datasets. It is entirely open source and freely available at https://scimodom.dieterichlab.org.

Sci-ModoM is underpinned by FAIR data principles, a standardized nomenclature, and interoperable formats including rich metadata[2], fostering the use of common standards within the epitranscriptomics community. It promotes data reuse by leveraging original published results, rather than re-processing raw data. But issues such as inconsistencies in dataset comparisons, e.g. arising because of a lack of standardized guidelines for reporting modifications using quantitative or stoichiometric information, are far from being solved. Our current work aims to expand the different RNA types (mRNA, non-coding RNA, tRNA, rRNA) in Sci-ModoM, to further establish FAIR data treatment, and to improve guidelines for data analysis and exchange, under the umbrella of the Human RNome project, thereby enhancing the utility and impact of latest discoveries.

[1]Etienne Boileau, Harald Wilhelmi, Anne Busch, Andrea Cappannini, Andreas Hildebrand, Janusz M. Bujnicki, Christoph Dieterich. Sci-ModoM: a quantitative database of transcriptome-wide high-throughput RNA modification sites Nucleic Acids Research, 2024, gkae972.
[2] https://dieterich-lab.github.io/euf-specs

S4-O17

Computational limitations and future needs to unravel the full potential of 2'-O-Methylation and C/D Box snoRNAs

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Epitranscriptomic research is gaining unprecedented momentum, prompted by the development of tools to fine-map RNA modifications. Ribose 2′-O-methylations (2′-O-Me) are the most abundant in ribosomal RNA (rRNA), where they act as major contributors to ribosome heterogeneity and translational control. Emerging evidence indicates that 2′Ome might be more widespread than previously recognized, potentially affecting also messenger RNAs stability and translation. 2′-O-methylations are deposited by small nucleolar ribonucleoproteins (snoRNPs) directed to the target site by C/D box small nucleolar RNAs (snoRNAs). Alterations of 2′-O-methylations and snoRNA levels are implicated in various diseases, including cancer, where they contribute to aberrant gene expression and tumorigenesis. Thus, identifying all snoRNAs associated with 2'-O-Me modifications is crucial for understanding their role in physiological and disease conditions.

Here we describe advantages and drawbacks of current C/D box snoRNA and 2′-O-Me databases and computational prediction tools. While they are great resources for characterizing C/D box snoRNAs and the 2′-O-Me sites they guide, they suffer from the quality and completeness of the data they rely on. In fact, no comprehensive collection of experimentally validated human snoRNA-guided 2′-O-Me sites currently exists, and these assignments are primarily derived through predictive analyses. This affects both database annotation and computational tools accuracy, as exemplified by discrepancies between predicted and functional snoRNA-guided methylation sites.

There is an urgent need for systematic experimental studies to refine predictions and improve database accuracy as well as for developing sophisticated computational approaches that provide comprehensive snoRNA annotations and predict novel guides associated with newly discovered 2′-O-methylations. Refining these approaches is critical to unraveling the functional complexity of 2′-O-Me in ribosome biology, cancer epitranscriptomics, and mRNA regulation. By bridging computational predictions with mechanistic validation, we can unlock the translational potential of 2′-O-Me as biomarkers and therapeutic targets.

S5-O18

Cell-free translation assays: corner-stone approaches to explore ribosome-based translation regulation

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The discovery that ribosome composition is dynamic has opened a new field in translation regulation, positioning the ribosome as a central regulatory entity. Translation is regulated by multiple factors, including mRNA features, translation factors, and ribosome-associated proteins and RNAs. Dissecting the specific contribution of the ribosome to translation regulation represents a significant challenge. Furthermore, altering the expression of ribosome biogenesis factors or ribosomal proteins can induce pleiotropic effects, complicating the attribution of observed translational changes directly to modifications in ribosomal composition. To study how ribosome structure relates to function, it is crucial to use functional assays that allow precise control over the translational machinery. Cell-free translation assays offer powerful tools by enabling controlled comparison of the intrinsic translational activity of different purified ribosome populations, independent of other cellular components. A hybrid method developed few years ago, consists of a cell-free assay to assess the translational activity of stringently purified human ribosomes. This method has proven effective for investigating the effects of different sources of ribosome heterogeneity, including rRNA modifications. However, the system relies on the highly specialized translational machinery provided by rabbit reticulocyte lysates, and measures ribosome-mediated protein synthesis through enzymatic activity of enzymes encoded by pre-defined reporter mRNA. These characteristics restrict the range of biological questions that can be addressed. Very recently, a tripartite cell-free translation system was implemented based on the separation of mRNAs, ribosomes and ribosome-depleted cytoplasmic lysate from human cells, allowing for flexible reconstitution of translation reactions. This system overcomes the main limitations of the hybrid system, and allows exploring the regulation of the translational machinery in controlled physio-pathological cellular contexts.

Here, we will provide an overview of the strengths and limitations of both approaches, and propose future developments and guidelines for their application to the study of ribosome heterogeneity.

S5-O19

rRNA Chemical Modifications as Dynamic Translational Regulators in Cancer and Chemoresistance

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Emerging evidence has redefined the ribosome as a dynamic regulator of gene expression. Within this paradigm, ribosomal heterogeneity, including rRNA chemical modifications, contributes to selective mRNA translation in response to various physiological and pathological contexts.

We first showed that modifications of rRNA methylation can occur during tumor initiation and progression. The modification of rRNA methylation pattern was associated with change in translational control of mRNAs encoding oncogenic proteins. In addition, analyses of human samples series issued from different cancer types allowed identification of components involved in ribosome biogenesis and rRNA methylation as independent markers of poor prognosis. In parallel we showed that, unexpectedly, rRNA chemical modifications can also occur following treatment with anti-cancer drugs such as the widely used chemotherapeutic drug 5-fluorouracil (5-FU).

I will show how we identified the direct chemical incorporation of 5-FU into rRNA and how this modification alters both global and mRNA-specific translation with implications for drug resistance mechanisms. I will discuss the limitations and the challenges we are facing to show how these “non-natural” rRNA chemical modifications profoundly alters the ribosome's functional landscape. I will discuss how redefining 5-FU not only as a DNA-damaging agent but also as a modulator of ribosome composition and function opens up a novel perspective for the development of molecules that have the potential to integrate within RNA and more particularly within rRNA.

Finally, I will discuss the limitations of the functional test we are using such as bi-cistronic assays, in vitro translation and translatome analysis to determine how modulation of rRNA chemical modifications affect translational efficiency of mRNAs promoting tumorigenesis and escape to anti-cancer treatments.

S5-O20

Workflows and methods for assessing impact of dynamic rRNA 2′-O-methylations on translation and cell phenotypes

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We have previously shown that alterations in rRNA 2′-O-methylation (2′-O-me) levels at specific key sites can influence ribosome function resulting in differential translation of mRNA transcripts, and that this can affect cell phenotypes. Here example workflows and methods used to assess functionality of selected rRNA 2′-O-me sites that display alterations in levels in response to internal and external stimuli will be discussed. This will include description of model systems used and application of methods such as RiboMeth-seq, assays for global translation, ribosome profiling, proteomics and further phenotypic analyses. Application of such methods and workflows will be demonstrated with real examples taken from our previously published and new unpublished data where regulation of ribosome function by candidate rRNA 2′-O-me positions has been explored in detail. These include translational regulation by a 2′-O-me site where dynamic alterations in methylation level were observed in response to expression of the c-MYC oncogene, the role of a particular rRNA 2′-O-me in regulating translation during cell differentiation to neuroectoderm lineages, and the impact of methylation level at another position on translation of transcripts belonging to distinct functional categories important in colorectal cancer.

S5-O21

Challenges in Linking snoRNA-Guided rRNA Modifications to Translation Regulation

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Small nucleolar RNAs (snoRNAs) guide chemical modifications on ribosomal RNA (rRNA), processes traditionally linked to ribosome biogenesis. However, emerging evidence suggests that these modifications may also regulate translation. Most rRNA modifications occur constitutively at conserved ribosomal core sites, ensuring structural stability and global ribosome function. In contrast, partially modified or variable sites may enable fine-tuned regulation of specific mRNAs, raising the question of how individual rRNA modifications influence translation dynamics. Addressing this requires careful analysis of polysome profiles to detect subtle shifts in translation across ribosome states (40S, 80S, and polysomes). Importantly, normalization across these fractions is essential to uncover biologically meaningful changes in translation efficiency, particularly for mRNAs sensitive to rRNA modification levels. Adding complexity, many snoRNAs are embedded within introns of host genes involved in ribosome biogenesis, creating regulatory dependencies that complicate functional studies. Knockdown approaches risk disrupting host gene function, confounding snoRNA-specific effects. To address these challenges, we compared antisense oligonucleotides (ASOs) and CRISPR-Cas13d approaches to knockdown SNORA81, a snoRNA associated with ovarian cancer and predicted to guide a partially modified pseudouridine site on 28S rRNA. Our results indicate that knockdown methods vary in their effects on host gene expression, with CRISPR-Cas13d showing off-target effects and ASOs affecting host gene regulation. We also compared different approaches to analyzing polysome profiles and their impact on interpreting translational effects. Notably, analysis of individual polysome fractions revealed defects in translation that were undetectable with pooled fraction comparisons. By using strict controls for host gene effects and employing robust normalization methods, we found that SNORA81 depletion selectively reduced ribosome association with GC-rich mRNAs involved in tumor pathways, without affecting global polysome profiles or ribosome abundance. These findings underscore the importance of integrating high-resolution mapping of snoRNA targets with refined polysome profiling approaches to uncover transcript-specific translational regulation.

S5-O22

Uncovering the impact of snoRNA-guided rRNA modification heterogeneity in a model of non-small cell lung cancer invasion

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The processing, folding, and chemical modification of ribosomal RNAs (rRNAs) are critical for proper ribosome assembly and function and are tightly coordinated by a complex network of protein and non-coding RNAs (ncRNA) assembly factors. Among these, an abundant group of ncRNAs, small nucleolar RNAs (snoRNAs), guide the folding and site-specific modification of over 200 nucleotides in human rRNAs. While these modifications are essential for efficient and accurate translation, their individual molecular functions remain poorly defined. This is further complicated by evidence that snoRNAs are implicated in additional gene regulatory processes, ranging from splicing to protein secretion, raising the need to uncouple their canonical roles in ribosome biogenesis from broader cellular functions. Further, snoRNA research is hindered by technical limitations in annotation, target prediction, and functional analysis without altering host gene context. These issues reduce reproducibility and complicate comparisons across systems, particularly in cancer models where gene expression and RNA processing are widely deregulated. To address these challenges, we leveraged advanced high-throughput sequencing and biochemical approaches in a cell culture model of collective invasion in non-small cell lung cancer (NSCLC). Using live cell image-guided genomics, we isolated spatially distinct tumor subpopulations for snoRNA expression and rRNA modification profiling, enabling us to define how snoRNA-guided translational control contributes to invasive behavior and cell-cell cooperation in NSCLC. Our findings reveal unique snoRNA expression profiles and rRNA modification patterns in distinct NSCLC subpopulations and show that these changes do not correlate with each other. Additionally, we demonstrate that these isolated cell subpopulations have differential translation capacities that can be altered by changing the expression of individual snoRNAs, dependent on their rRNA base-pairing ability. Collectively, our data advance our understanding of ribosome-level control in collective invasion and underscore the need for standardized tools and annotations to enable systematic study of translation regulation in cancer.

S6-O23

Discriminating Between Catalytic and Non-Catalytic Functions of snoRNPs

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Small nucleolar ribonucleoproteins (snoRNPs) are central players in ribosome biogenesis, typically composed of a guide snoRNA and four core proteins. The snoRNA base-pairs with pre-rRNA to direct site-specific modifications catalyzed by a core protein. In yeast, box C/D snoRNAs guide 2'-O-methylation through Nop1 (fibrillarin in humans), while box H/ACA snoRNAs such as snR37 guide pseudouridylation via Cbf5 (dyskerin in humans).

In addition to their catalytic roles, snoRNPs can influence rRNA folding and structure. Despite this fact, defects or alterations resulting from snoRNA deletion have often been interpreted solely as consequences of modification loss. Here, we show that such interpretations can be misleading. Notably, with few exceptions, individual deletion of most yeast snoRNA genes - including SNR37 - does not result in any growth defects. To sensitize yeast cells to the absence of SNR37, we searched for mutants that cause growth defects in its absence. We then exploited the identified genetic interactions with ribosome assembly factors to test the functionality of snr37 mutants, aiming to dissect the dual roles of the snR37 snoRNP by selectively impairing either its modification or non-catalytic functions. Of note, while some of the phenotypes observed for an snr37Δ strain match the defects arising upon loss of pseudouridylation, others are independent from pseudouridylation, likely reflecting the disruption of structural functions.

Our findings highlight the need to consider both catalytic and non-catalytic snoRNP functions when interpreting deletion phenotypes, and offer strategies for disentangling these roles.

S6-O24

RNA binding by H/ACA snoRNPs – near-cognate interactions beyond pseudouridine formation

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H/ACA snoRNPs recognize target RNAs for pseudouridylation through base-pairing interactions. We and others have previously defined the minimal interactions required to identify a uridine for modification which includes a minimum of 8 base-pairs and at least 3 base-pairs on each site of the target uridine. Thus, we understand well how target uridines are selected by H/ACA snoRNPs.

However, we made the surprising experimental observation that H/ACA snoRNPs also bind tightly to sequences that are not modified, but that can form a significant number of base-pairs with the pseudouridylation pocket in H/ACA snoRNA. We call these near-cognate interactions and have shown that they can strongly compete with cognate interactions at pseudouridylation sites.

The observation of near-cognate interaction sites for H/ACA snoRNPs raises several questions on the biological functions of H/ACA snoRNPs and possibly overlooked interaction sites.

To begin addressing these questions, we asked whether such near-cognate interactions are common or whether they are selected against in evolution. Notably, we were able to predict many near-cognate interaction sites in ribosomal RNA for yeast H/ACA snoRNAs which may suggest that these are positively selected for in evolution for functional reasons.

Such near-cognate interactions of H/ACA snoRNPs likely have biological functions. Possibly, near-cognate sites may serve as landing sites for H/ACA snoRNPs while searching for cognate sites. Additionally, near-cognate interactions with H/ACA snoRNPs may contribute to keeping RNA unfolded, e.g. during the early stages of ribosome biogenesis.

S6-O25

H/ACA snR30 snoRNP guides independent 18S rRNA subdomain formation

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The initial phases of eukaryotic ribosome assembly, and in particular the role of small nucleolar RNAs (snoRNAs) in guiding pre-rRNA folding and modification, remains poorly understood. A significant challenge is the structural visualization of these transient and dynamic early intermediates. Despite the advances made in cryo-electron microscopy (cryo-EM), many snoRNA-mediated mechanisms remain enigmatic. An example of this is snR30 (U17 in humans), the only essential H/ACA snoRNA in yeast, whose exact role in 40S subunit biogenesis has long been unclear considering the lack of associated rRNA base modification. Utilizing cryo-EM in combination with a dominant negative Krr1 trapping mutant, we reveal the structure of snR30 bound to the H/ACA core proteins (Cbf5-Gar1-Nop10-Nhp2) and to a specific subdomain of the pre-18S rRNA on a stalled 90S particle. This domain comprises central domain helices h20–h23 and part of expansion segment 6 (ES6). As chaperoned by the snR30 snoRNP, the Central domain remains externalized from the evolving 90S moiety. The snoRNP contains, in addition, the early assembly factors (Krr1, Utp23, Kri1) and ribosomal proteins (uS11, uS15), forming a bimodular complex containing the H/ACA core and the 18S platform module. Subsequent Krr1-mediated dissociation of snR30 eventually enables full integration of this domain into the pre-ribosome. This work gives insides into how non-catalytic snoRNAs choreograph local rRNA architecture to facilitate ribosome assembly. Furthermore, it emphasizes the issue of capture and interpretation of highly dynamic intermediates by cryo-EM. Here, the combination of cryo-EM with biochemical trapping and time-resolved strategies may provide new approaches for the visualization of these fleeting states. By revealing a checkpoint-like function for snR30, we highlight how modular assembly principles underlie early ribosome biogenesis and how cryo-EM can go beyond static structures to illuminate RNA-guided assembly.

S6-O26

Mapping RBP-specific snoRNA interactions with chimeric eCLIP

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Small nucleolar RNAs (snoRNAs) are non-coding RNAs that function in ribosome and spliceosome biogenesis, primarily by guiding modifying enzymes to specific sites on ribosomal RNA (rRNA) and spliceosomal RNA (snRNA). However, many orphan snoRNAs remain uncharacterized, with unidentified or unvalidated targets, and studies suggest that many additional RNA binding proteins may interact with snoRNAs for non-canonical RNA processing roles. To address this challenge, we developed an enhanced chimeric eCLIP approach to comprehensively profile snoRNA-target RNA interactions using both core and accessory snoRNA binding proteins as baits. Using core snoRNA binding proteins, we confirmed most annotated snoRNA-rRNA and snoRNA-snRNA interactions in mouse and human cell lines and called novel, high-confidence interactions for orphan snoRNAs. While some of these interactions result in chemical modification, others may have modification-independent functions. We then showed that snoRNA ribonucleoprotein complexes containing certain accessory proteins, like WDR43 and NOLC1, enriched for specific subsets of snoRNA-target RNA interactions with distinct roles in ribosome and spliceosome biogenesis. Notably, we discovered that SNORD89 guides 2'-O-methylation at two neighboring sites in U2 snRNA to fine-tune splice site recognition. Thus, chimeric eCLIP of snoRNA-associating proteins enables a comprehensive framework for studying snoRNA-target interactions in an RNA binding protein-dependent manner, revealing novel interactions and regulatory roles in RNA biogenesis.

• Abstracts Poster Presentations •

P1

Optimizing snoRNA Sequencing: Comparative Analysis of Reverse Transcriptases and Library Preparation Approaches

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Small nucleolar RNAs (snoRNAs) are critical regulators of ribosomal RNA modification and ribonucleoprotein complex assembly and are increasingly linked to human diseases, including cancer and neurodegeneration. Despite their importance, the compact size and structured nature of snoRNAs pose significant challenges for accurate quantification using standard RNA sequencing methods. To address these limitations, we conducted a systematic evaluation of five reverse transcriptase enzymes, Induro (NEB), TGIRT (Ingex), SuperScript IV (ThermoFisher), Maxima H Minus (ThermoFisher), and SEQzyme (Bio-Rad), to identify optimal conditions for snoRNA sequencing.

Using two human cell lines and synthetic mouse snoRNA spike-ins as internal controls, we assessed sensitivity, bias, and reproducibility across enzymes and library preparation strategies. The synthetic spike-ins, which lack sequence similarity to human transcripts, provided a robust normalization framework for cross-method comparisons. We tested multiple library construction approaches, including ligation-based and template-switching methods, alongside various size-selection protocols. Key performance metrics included processivity, fidelity, coverage uniformity, and compatibility with highly structured RNAs.

Our results revealed significant differences in enzyme performance and highlighted the impact of library preparation strategies on snoRNA recovery. Notably, template-switching methods showed improved recovery of structured RNAs compared to ligation-based approaches in certain conditions. The incorporation of synthetic spike-ins proved essential for benchmarking methods and minimizing biases introduced during sequencing.

This study establishes a foundation for optimizing snoRNA sequencing workflows and emphasizes the importance of selecting appropriate sequencing strategy when studying structured noncoding RNAs.

P2

Integrated snoRNA Profiling at the U. Sherbrooke RNomics Platform: Sequencing, Validation, and Quality Assurance

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The RNomics Platform at Université de Sherbrooke offers end-to-end solutions for small nucleolar RNA (snoRNA) detection and validation, combining next-generation sequencing with RT-qPCR and ddPCR technologies. snoRNAs, critical for rRNA modification and linked to diseases like cancer, pose detection challenges due to their small size, high structure, and low abundance. Our platform addresses these challenges through a rigorously validated workflow. Sequencing begins with total RNA isolation from cell or tissue samples, followed by quality control using Agilent Bioanalyzer/Tape Station or Perkin Elmer GX Touch (RIN ≥8) and quantification via Qubit fluorometer (Invitrogen). To enhance snoRNA detection, we perform ribosomal RNA depletion using hybridization-based probes (e.g., riboPOOLs (siTOOLs Biotech)), with depletion efficiency verified by qPCR (or ddPCR) and Bioanalyzer traces to ensure <5% residual rRNA. Libraries are prepared using in house snoRNA sensitive library prep protocol optimized for structured RNAs. Sequencing on Element Bioscience's Aviti™ to achieve high coverage of snoRNAs, with synthetic spike-ins (e.g., mouse snoRNAs) enabling cross-sample normalization and bias detection. For validation, candidate snoRNAs are quantified via RT-qPCR (Bio-Rad Opus 384/96) or absolute ddPCR (Bio-Rad QX200), using platform-designed primers validated for specificity and efficiency. Bioinformatics pipelines process sequencing data, mapping snoRNAs to annotated databases (e.g., snoDB) and filtering artifacts via read alignment and secondary structure prediction. Platform services emphasize reproducibility, with technical replicates and inter-assay controls standard in all workflows. By integrating sequencing, validation, and stringent quality controls, the RNomics Platform provides researchers with robust tools to explore snoRNA roles in disease, supported by customizable bioinformatics and expert consultation.

P3

Addressing Annotation Gaps in the Mouse snoRNome and rRNA 2'O-Methylation Landscape

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C/D box small nucleolar RNAs (SNORDs) were discovered about 50 years ago, with their role in 2'-O-ribose methylation (2'Ome) of ribosomal RNA (rRNA) identified two decades later[1]. In vertebrates, snoRNAs are often encoded within introns, exist in multiple copies, and have complex secondary structures, complicating their annotation and quantification[1,2]. While human snoRNAs and rRNA 2'Ome sites are well-characterized and cataloged in databases such as snoDB, efforts are now extending to other organisms[3,4].

Beyond their canonical functions, snoRNAs were shown to target non-canonical substrates and to play roles in additional physiological[1]. They also are implicated in pathologies such as cancer and neurological disorders[5]. However, in mice—a key model in biomedical research—snoRNA annotations remain incomplete and inconsistent, as does the cartography of rRNA 2'Ome. Thus, an exhaustive list of mouse SNORDs and rRNA 2'Ome sites are still lacking: of the 1,508 snoRNAs listed in the mm39 annotation, only 113 are clearly identified as C/D-box based on their gene description or name—limiting their utility for comparative and functional studies.

To address these gaps, we aim to systematically annotate murine SNORDs. Using four computational predictors on the mm39 assembly, we generated 1.75 million predictions and got down to 750,000 predictions after filtering, with 36,653 high-confidence candidates shared across multiple predictors. Expression profiling of these 36,653 candidates across ten tissues using the TGIRT-CoCo pipeline —optimized for snoRNA quantification[2]— identified 614 expressed predictions. Further validations are ongoing, including replication on two additional mouse strains. To establish a comprehensive mapping of 2'Ome sites on murine rRNAs, we will then predict snoRNA-rRNA interactions and validate identified sites in RiboMethSeq datasets of the same samples used for SNORDs expression profiling.
This work refines murine snoRNA and 2'Ome annotation and nomenclature, lays ground for functional studies of murine SNORDs and rRNA 2'Ome, and enables more robust cross-species comparisons.

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P4

Investigating the proteins involved in the FUS-mediated biogenesis of sdRNAs - small RNAs derived from snoRNAs

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The variety of non-coding RNAs is still growing, including an emerging class of small RNAs called sdRNA. These are stable RNAs 25-35 nucleotides long, derived from snoRNAs (small nucleolar RNAs), which may function as microRNAs and regulate gene expression. However, the mechanism of sdRNAs generation in the cell is not yet fully understood.

Recently, in cells with FUS protein depletion (FUS KO), we have observed differential expression of numerous snoRNAs followed by an altered level of the corresponding sdRNAs, suggesting FUS involvement in this process. FUS is a multifunctional protein involved in many pathways of RNA metabolism. It is localized mainly in the cell nucleus, however, there are mutations in the FUS gene that trap the FUS protein in cytoplasmic aggregates. These mutations have been associated with the neurodegenerative disease amyotrophic lateral sclerosis (called ALS-FUS hereafter). In cellular models of ALS-FUS, we have observed changes in the level of snoRNAs and sdRNAs as well.
To elucidate the molecular mechanism of FUS-mediated sdRNA biogenesis, we first performed RNA antisense purification (RAP) of selected snoRNAs from protein extracts of SH¬-SY5Y WT and FUS KO cells followed by mass spectrometry to identify snoRNA-interacting proteins. This resulted in the selection of RALY, ILF3, DDX27 and DHX30 proteins, as potential candidates that participate in sdRNA biogenesis together with FUS. Next, we confirmed these interactions by IP (FUS:proteins) and tested the effect of FUS on these proteins expression (at the mRNA and protein level) by RT-qPCR and Western Blot. Moreover, we analyzed how the FUS:snoRNA and FUS:protein interactions were affected by ALS-FUS mutations, FUS P525L and FUS R495X using CRISPR-edited SH-SY5Y cells. Furthermore, due to the pathological cytoplasmic phenotype of ALS-FUS, we tested the subcellular localization of selected proteins by immunofluorescence.

The results obtained enable us to indicate a novel protein(s) that interact with FUS and participate in the processing of snoRNAs into sdRNAs. It will bring us closer to the mechanism of sdRNA biogenesis mediated by FUS and its role in the pathology of ALS.

P5

Functional characterization of snoRNA-derived RNA expression in healthy hematopoiesis and acute myeloid leukemia

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SnoRNAs are highly expressed in cancers including acute myeloid leukemia. SnoRNAs can be further processed into snoRNA-derived RNAs (sdRNAs). The expression patterns and functional implications in acute myeloid leukemia and healthy hematopoiesis, however, remain largely elusive. We characterized sdRNA and snoRNA levels in hematopoietic stem cells (HSCs), healthy white blood cells (WBCs) of lymphoid and myeloid differentiation, as well as in 159 samples from intensively treated AML patients at initial diagnosis. Out of 927 snoRNA loci with assigned read counts, 135 served as sdRNA hostgenes. Of these, 72 snoRNAs produced multiple sdRNAs, resulting in a total of 212 distinct sdRNAs. The majority originated from the 3'-end (sd3'-RNAs, 114/212), while the 5'-end-derived sdRNAs accounted for 38,2% (sd5'-RNAs, 81/212), and only a small fraction (17/212, 8,0%) were derived from the central region (sdx-RNA) of the respective host snoRNA. Even though sd3'-RNAs represented just 53,8% of the total sdRNAs identified, they made up more than 85% of total sdRNA reads across all samples.

We showed that HSCs, healthy WBCs and AML blasts can be differentiated by their sdRNA expression pattern in a cell-type specific manner. In AML, we observed high sd3'-RNA/snoRNA-hostgene ratios to be associated with inverse patient outcome. Particularly, in NPM1-mutated patients with favorable risk stratification (ELN) and good initial therapy response, high sd3'-RNA ratios identify a subgroup with significantly worse outcome. Transcriptome analyses revealed a clear association of high sd3'-RNA ratios in NPM1-mutated AML with alterations in oncogenic and immune response signaling. Functionally, forced expression of single sdRNAs can enhance clonogenic potential in AML.
Our data showed that at least three types of sdRNAs exist, defined by their prior relative position within their snoRNA hostgene, and that differential sdRNA subtype expression has vast implications in healthy hematopoiesis and AML. We therefore postulate an sdRNA nomenclature clearly indicating the relative position within the snoRNA hostgene (sd3'-RNAs, sd5'-RNAs, sdx-RNAs).

P6

Identification and characterisation of snoRNAs as circulating biomarkers of bone recurrence in breast cancer

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Bone metastases occur in 80% of patients with advanced breast cancer and represent a major challenge in oncology due to their detrimental effects on bone homeostasis and patient quality of life. Therefore, better understanding the underlying mechanisms and identifying new predictive biomarkers is essential to improve patient management. We propose an innovative approach by studying small nucleolar RNAs (snoRNAs), which are non-coding RNAs guiding the chemical modification of ribosomal RNAs (rRNAs), particularly 2'O-methylation (2'Ome). Alterations of rRNA 2'Ome have been observed in various cancers (e.g., breast and glioma), promoting the translation of specific mRNAs and acquisition of cancer cell characteristics. Using the AZURE cohort (BIG 01/04), we quantified 39 circulating snoRNAs in the serum of 48 patients with early-stage breast cancer. A panel of 12 snoRNAs was significantly associated with bone recurrence. Moreover, some of those snoRNAs were found to be under-expressed in ALDHhigh breast cancer cells with high metastatic potential and correlated with circulating biomarkers of bone homeostasis. Based on these preliminary results, we hypothesize that altered snoRNA expression in breast tumor cells affects rRNA 2'Ome, leading to translational deregulation that promotes bone metastatic progression.

P7

Snogging with the introns: challenges in studying maturation and snoRNP composition in avian cells.

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Erasmus MC

The technical challenges we wish to share with the snoRNA community concern an underutilized platform for snoRNA studies: avian cells. We aim to: (i) monitor by qPCR mature and immature forms of snoRNAs in chicken cells under physiological conditions, upon vector-based overexpression, or during avian Influenza A virus infection (AIV), and (ii) pulldown chicken snoRNAs toward snoRNP identification via mass-spectrometry. Although repeatedly successful, snoRNA overexpression and pulldown standardizations in human cells may account for the below-mentioned discrepancies we observed in chicken cells.

The importance of studying avian snoRNAs stems from earlier findings of our group (Gultyaev et al., 2021), suggesting snoRNA involvement in Highly Pathogenic Avian Influenza Virus (HPAIV) genesis, occurring primarily in terrestrial poultry. HPAIVs present a serious threat to avian health and agribusiness, owing to near-total fatality in infected populations. HPAIVs are characterized by an acquired multi-basic cleavage site in the hemagglutinin surface glycoprotein. In nature, this can occur through non-homologous recombination of the hemagglutinin RNA with host or viral RNA. We identified snoRNA binding sites in the inserted sequences near the recombination points, prompting us to investigate the expression and functions of these snoRNAs during AIV infection in chicken cells.

We unexpectedly found increased immature snoRNA levels, upon SNORD49, SNORD21, SNORA22, or SNORA68 overexpression using a cassette comprising natural chicken sequences with flanking exons under a CMV promoter. RT-PCR analysis revealed proper exon-exon splicing during overexpression. However, we additionally detected puzzling intermediate exon-snoRNA fragments with both upstream or downstream exons in transfected and control cells. Whether these findings could illuminate the snoRNA maturation process in avian cells requires further examination. We are currently confirming them by northern blot as an alternative approach. Furthermore, we plan to outline different experimental designs and controls used in snoRNA pulldowns, fostering discussions and sharing our perspectives toward standardizing in vivo protocols.

P8

Functional landscape of human multicopy SNORD genes at DNA and RNA level

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Besides their canonical role some box C/D small nucleolar RNAs (SNORDs) are suspected to regulate several levels of gene expression, while around a third of the 1,000 or so human SNORD genes are currently functionally orphan. Here, we present recent findings that SNORDs can act at DNA and RNA levels and that decrypting this information can help us better understand their function. We first focused on multigenic SNORD families associated with pathologies, e.g. rare neurological diseases such as Prader Willi syndrome for the SNORD115 and SNORD116 genes or Labrune syndrome for the SNORD118 genes. First, as already described for other gene categories, we collected evidences of neofunctionalization of several SNORD copies. Second, we found that non-allelic gene conversion events promote genetic homogeneity between paralog copies, particularly when they are genetically colocalized, and propagate single-nucleotide polymorphisms that can be pathological. Third, we observed variable rates of SNORD gene gains and losses with potential consequences for gene dosage; surprisingly, the variation in copy number exhibits evidences of coordination for the neighboring tandem repeats SNORD115 and SNORD116 in several eutherian species, which could be due to a differential chromatin compaction level of parental genomes caused by genomic imprinting. Fourth, the analysis of paralog and ortholog sequences can identify selective constraints at nucleotide level, including in human populations, which represents a powerful tool for functional study. As a leading example, a recent characterization of highly conserved hybridization capacities with RNA targets has opened up a new avenue concerning the molecular function of the SNORD116 family, the absence of which is strongly suggested to be critical in Prader Willi syndrome. In conclusion, our intention with this work is to sugget that, in contrast to a static view, the function of several members of this class of non-coding RNAs is dynamic, composite and emerges from multiplicity.

P9

The DLK1-DIO3 C/D-box snoRNA locus in cardiovascular disease; Genetic associations, plasma levels and fibrillarin-dependent 2'O-ribose methylation of NON-ribosomal RNAs

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The imprinted DLK1-DIO3 locus on human chromosome 14 (14q32) encodes 41 C/D box snoRNAs, SNORD112, SNORD113 (1-9) and SNORD114 (1-31), that are located in between the MEG3 lncRNA gene and a cluster of 54 microRNAs, which have been reported to play a role in the development and progression of human cardiovascular disease. We found that single nucleotide polymorphisms (SNPs) in the snoRNA-cluster were significantly associated with cardiovascular diseases, including heart failure. These SNPs were not linked to SNPs in the microRNA-cluster or in MEG3, indicating that snoRNAs modify the risk of cardiovascular disease independently. In accordance with these findings, 14q32 snoRNAs showed increased expression in various diseased cardiovascular tissues compared to healthy human and murine tissue samples. Furthermore, 14q32 snoRNA expression was up-regulated in various primary human vascular cell- and tissue-cultures in response to pathological culture conditions, such as hypoxia and ischemia, in vitro and ex vivo.

Initial experiments showed that 14q32 snoRNAs bind predominantly to fibrillarin, indicating that they act through canonical mechanisms, but on non-canonical RNA targets. We aimed to identify these targets, focusing on the CVD-associated SNORD113-6 (AF357425 in mice). As we found that AF357425-knockout cells were non-viable, we induced over-expression or inhibition of AF357425 in primary murine fibroblasts. We pulled-down fibrillarin from AF357425-High versus AF357425-Low fibroblast lysates, followed by RNA isolation, ribosomal RNA depletion and long and small RNA-Seq. We identified both (pre-)mRNA and tRNA targets, both of which appeared stabilised and protected from degradation/ cleavage at their respective 2'Ome sites. These targets were conserved in murine and human cells. Among the mRNA targets, we observed enrichment of the integrin signalling pathway, which is crucial for vascular integrity. Indeed, inhibition of SNORD113.6 directly impacted primary human arterial fibroblasts' barrier function, as well as fibroblast-induced tissue contraction.

Even though 2'Ome could only be determined via indirect methods, due to low target RNA abundance and ultra-short sequences, our findings merit increased effort and attention to non-ribosomal snoRNA targets.

P10

Beyond rRNA modification: the unconventional life of the snR37 snoRNP

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The maturation of pre-rRNAs has an impact on the entire ribosome assembly, starting directly after rDNA transcription with extensive processing, modification and folding events. In highly flexible pre-ribosomal particles pre-rRNA modifications are introduced by small nucleolar nucleoprotein complexes (snoRNPs). H/ACA box snoRNPs are guided by snoRNAs that typically are around 200 nucleotides in size, form two distinct hairpin structures and carry two sets of four conserved core proteins. However, a peculiar H/ACA box snoRNA, snR37, not only possesses an extraordinary length of 386 nucleotides, but also includes three additional core proteins, novel ribosome assembly factors Upa1, Upa2 and Rbp95. Due to its unique features snR37 offers specific RNA elements, which are revealed in the cryo-EM structure of snR37 snoRNP at a 2.8 Å resolution. We demonstrate that Upa1 and Upa2 are required for the efficient binding of snR37 to pre-60S particles, whereas base-pairing of snR37 with the rRNA target, the 25S rRNA, is not required for association. Furthermore, Upa1, Upa2, Rbp95 and snR37 are genetically linked to the Npa1 complex, which contains characteristic assembly factors of early pre-60S particles. snR37 variants incapable of pseudouridylation at U2944 can fully rescue a synthetic growth defect caused by the deletion of snR37. Hence, we propose that snR37 has an important function beyond rRNA modification, serving as a scaffold for rRNA folding in the early steps of pre-60S maturation.

P11

The variant La protein Mlp1 is a snoRNA binding protein that influences U4/U6 di-snRNP assembly and splicing in Tetrahymena thermophila

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La and La-related proteins are linked to a number of RNA-associated processes, including snoRNA guided 2′-O-methylation. La proteins typically rely on an N-terminal La motif and RRM to bind RNA polymerase III transcripts, but the variant Tetrahymena thermophila La protein Mlp1 targets and influences La-associated pre-tRNAs in the absence of the classical RRM. Predicted structural characterization of Mlp1 suggests the presence of a variant xRRM domain in place of the RRM that may be linked to new functions and RNA targets not typically associated with the genuine La proteins. However, the relatively lower annotation of snRNAs and snoRNAs in this otherwise highly studied model organism has limited analysis of Mlp1 function for such non-coding RNAs in this system.

We have performed Mlp1 fPAR-CLIP-seq from T. thermophila cells and identified a breadth of Mlp1 RNA targets, including direct associations with all of the mature snRNAs. In vitro electrophoretic mobility shift assays suggest that Mlp1 preferentially associates with the U4/U6 di-snRNA through an unconventional La protein binding mode. Additionally, Mlp1 partial knock out in this system influences the assembly of the U4/U6 di-snRNP and splicing of some mRNAs. Normal di-snRNP assembly often relies on snoRNA guided 2′-O-methylations, and our data also suggests that Mlp1 directly contacts all characterized CD (and H/ACA) snoRNAs in T. thermophila, hinting at a potential role of Mlp1 in the core snoRNP. Our findings support a model in which the altered domain architecture of Mlp1 underlies novel, ciliate-specific La protein functions.

P12

Parallelised high-throughput library preparation for profiling 2′-O-Methylations

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Recent evidence suggests that ribosomes are dynamic and heterogeneous machines, actively involved in translation regulation. This heterogeneity arises from many molecular interactors and epi-transcriptional and translational layers of molecular diversity, including rRNA modifications. 2′O-methylations (2′-O-Me) are the most widespread rRNA modifications, capable of influencing ribosome biogenesis and translation fidelity, potentially adding an extra layer of translation regulation. Among the most used techniques for high-throughput analyses and quantification of rRNA methylation levels is RiboMethSeq, a next-generation sequencing-based technique to resolve the rRNA 2′-O-Me landscape at single nucleotide resolution. Currently, there are no commercial kits specifically dedicated to this purpose, but several library preparation methods have been used, potentially yielding different results and influencing the biological interpretation of the data. In the framework of a collaboration with Immagina Biotechnology we aimed at developing a dedicated library preparation kit for parallelized high-throughput RiboMethSeq.

First, we evaluated whether LaceSeq (Immagina Biotechnology) is compatible with RiboMethSeq and yields results comparable to two other small RNA library preparation methods. A key advantage of LaceSeq is the presence of unique molecular identifiers that allow the removal of PCR duplicates during data analysis, an aspect not addressed by conventional methods. We silenced Snord93, responsible for guiding 18S:Am578, in NSC34 and compared the methylation scores obtained with the three methodologies. Our results show that methylation levels vary depending on the library preparation method used, and that not all methods are able to detect downregulation at 18S:Am578 upon Snord93 silencing. LaceSeq stands out as the library preparation method with the widest dynamic range, showing a 30.7% decrease in 18S:Am578, compared to a 13.1% decrease and an opposite 19.2% increase obtained with the other two technologies. Second, we developed a dedicated library preparation protocol for parallelized high-throughput RiboMethSeq combining LaceSeq with proprietary barcoded adaptors, streamlining multi-sample analysis and enabling RiboMethSeq clinical application for the identification of epitranslational-based biomarkers and therapies.

Overall, our results underscore the reliability of LaceSeq for comprehensive profiling of the 2'-O-Me landscape Furthermore, we are currently expanding its applicability to challenging low-input samples, increasing the versatility of the kit.

P13

Site-Specific Analysis of Ribosomal 2′ O-Methylation by Quantitative Reverse Transcription PCR Under Low Deoxynucleotide Triphosphate Concentrations

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Ribose 2′O-methylation (Nm, ribomethylation) is the most abundant RNA modification present in rRNA. It has been shown that alterations in ribosomal 2′O-methylation at individual Nm sites likely reflect regulated cellular processes. Although several analytical approaches for Nm detection and profiling have been developed, a simple and affordable method for the screening and measurement of individual Nm sites in large numbers of tissue samples is required to examine their potential for clinical translation. Here, we describe a new quantitative reverse transcription PCR-based method that can sensitively assess ribomethylation levels at specific rRNA sites at single-nucleotide resolution in low input amounts of total RNA.

P14

Bridging the Gaps in Ribosomal Pseudouridylation Levels Mapping by coordinating Chemical Reactivity and Direct Signal Perturbation-Based Quantifications

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Current methods for mapping rRNA pseudouridylation (Ψ), essentially chemical reactivity-based approaches (e.g., HydraPsiSeq) and signal perturbation profiling (nanopore sequencing), face complementary limitations that hinder accurate stoichiometric quantification. HydraPsiSeq quantifies Ψ levels via hydrazine resistance, offering precision for abundant modifications (>60% stoichiometry) but suffers from batch variability (σ2 =20%) due to RNA structural interference. Conversely, nanopore sequencing detects Ψ through ionic current disruptions, achieving single-molecule resolution, yet encounters difficulties with low-probability modifications (<10%) and requires thresholds and prior knowledge of the sites to filter modifications after basecalling. Our preliminary analysis revealed that HydraPsiSeq tends to overestimate hypomodified positions (e.g., <30% stoichiometry) up to a 2.5-fold factor compared to targeted orthogonal validation (Ψ-CMC-RT-PCR). Nanopore, while more consistent (CV <5%), struggles to resolve densely modified regions due to signal overlaps.

Integrating both methods addresses these gaps: HydraPsiSeq provides absolute quantification for highly modified sites, while nanopore captures structural dependencies and rare subpopulations. For example, in SNORA81-associated ovarian cancer models, combined analysis revealed GC-dependent translation linked to Ψ stoichiometry gradients (20–60%) undetectable by either method alone. Thus, standardization remains critical, nanopore requires environment-aware basecallers trained on cellular RNA, and HydraPsiSeq may benefit from rRNA fragmentation to mitigate accessibility biases. These advances are critical for cancer research, where dysregulated Ψ sites (e.g., 18S rRNA hypomodification of uridine 608 in liver cancer) drive oncogenic translation.

Here we reconcile chemical and biophysical detection in different cell systems for dynamic Ψ mapping by nanopore sequencing with: (1) comprehensive site coverage in regard to known pseudouridines in rRNA, (2) consistent precision in repeated measurements, and (3) sensitivity to subtle stoichiometric changes in a dilution range, relying on HydraPsiSeq as a safeguard. This integrated approach will certainly resolve lingering discrepancies in snoRNA studies and help to elucidate how rRNA modification heterogeneity fine-tunes translational programs in disease.

P15

Identification and validation of ribosomal RNA modifications in Caenorhabditis elegans - challenges and solutions

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Our overarching goal is to investigate whether ribosomal RNA modifications change throughout organismal aging in the nematode worm Caenorhabditis elegans. However, a comprehensive map of different types of modifications, including 2-O-ribose methylations (2'-O-m), pseudouridines (Ψ), and base modifications, is still unavailable. Thus, we first aimed to identify and validate modified sites in C. elegans rRNAs, a crucial step before progressing to comparative studies.

We performed Oxford Nanopore direct RNA sequencing after poly-adenylation of total RNA isolated from adult N2 wildtype worms and in vitro transcribed (IVT) 18S and 26S rRNA. This emerging technology allows the simultaneous detection of different types of RNA modifications and the identification of concerted modification changes at single molecule resolution. With stringent cutoffs to increase confidence in the identified sites, our analysis revealed ~100 modified positions in 18S, 26S, and 5.8S rRNA. Interestingly, a comparison with direct RNA sequencing data from human keratinocytes revealed that many identified sites and their characteristic error signatures are also present in humans. This finding suggests the evolutionary conservation of these modification sites. We used RiboMeth-seq and primer extension as orthogonal methods to validate the identified sites. Moreover, we depleted worm orthologues of known RNA-modifying enzymes for additional validation and compared changes in rRNA modification patterns between ad libidum-fed and dietary-restricted animals.

To conclude, we have generated a comprehensive map of different types of rRNA modifications in the important model organism C. elegans. With its stringent cutoffs and validation with other methods, this work provides a solid foundation for future research. While we acknowledge that we might have missed several positions, especially those that are only partially modified, we are excited about the potential impact of our findings on future studies that aim to correlate age- and nutrition-related changes in rRNA modifications with translation dynamics and physiology.

P16

Towards the creation of a comprehensive map of pseudouridine in rRNA & H/ACA box snoRNAs in Drosophila melanogaster

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Ribosomal RNAs (rRNAs) undergo chemical modifications from early transcription to maturation. This extensive array of RNA modifications is involved in ribosome biogenesis, ensuring the proper folding of the secondary and tertiary structures of the rRNA scaffold. Moreover, alterations in rRNA modification patterns and small nucleolar RNAs (snoRNAs) expression can affect development and contribute to genetic diseases and cancer. The two most abundant RNA modifications in rRNA are 2′-hydroxyl ribose moiety (Nm) and pseudouridine (Ψ). In eukaryotic rRNA, Ψ is introduced by the pseudouridine synthase Dyskerin, guided by H/ACA box snoRNAs. We aim to create a comprehensive map of Ψ in rRNA and H/ACA box snoRNAs in Drosophila melanogaster. Ψ mapping will be carried out by three different methods including HydraPsiSeq, BID-seq, and Nanopore, while corresponding H/ACA box snoRNAs will be detected using Induro-seq. So far, we have identified 79 Ψ sites with HydraPsiSeq, and we have validated the expression of 126 H/ACA box snoRNAs. We will investigate the dynamics of Ψ across development and under environmental stress. This map of Ψ and H/ACA box snoRNAs will pave the way for further exploration of the regulation of translation in response to cellular stress and disease in a whole organism.

P17

High-Resolution Mapping of RNA Modifications Reveals Mechanisms and Functional Impacts Across RNA Classes

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RNA molecules undergo a wide array of chemical modifications—collectively known as the epitranscriptome—which regulate RNA structure, stability, and function. To enable systematic investigation of these modifications, we have developed and applied next-generation sequencing-based technologies for high-throughput, single-nucleotide resolution mapping of RNA modifications. Focusing on RNA acetylation, 2′-O-methylation, and pseudouridylation, we profiled their distribution across rRNA, tRNA, and mRNA, and uncovered key enzymatic and structural determinants of their deposition. We identified an evolutionarily conserved RNA acetylation mechanism that enhances RNA thermostability. Our analysis revealed that the methyltransferase MRM2 specifically modifies mitochondrial rRNA, and that its loss disrupts mitoribosome assembly and compromises mitochondrial homeostasis. Furthermore, we discovered that snoRNAs, typically involved in rRNA modification, can be repurposed to guide pseudouridylation of mRNA. These findings reveal core regulatory roles of RNA modifications in cellular function and provide new mechanistic and design insights into RNA-targeting pathways, particularly snoRNA-mediated pseudouridylation.

P18

Advancing snoRNA-Guided Ribosome Heterogeneity: Overcoming Challenges for Functional Insights

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The snoRNA-guided ribosome heterogeneity gained significant attention over the last decade, as several seminal papers demonstrated its tight regulation and profound implications for ribosome function during development, (patho)physiological cellular processes and disease. Furthermore, a shift towards non-coding RNA (ncRNA) research coupled with the advances in (small)RNA-Seq methodology and ncRNA annotations resulted in a growing number of researchers reporting snoRNA dysregulation in their datasets. However, the follow-up on results is lagging.

Several factors hinder the functional analysis of snoRNA-guided ribosome heterogeneity, including the lack of standardisation of the complex nomenclature for snoRNAs, rRNA modification sites, ribosome helices within and across species, report practices, and reference sequence discrepancies (e.g. two distinct 28S reference sequences used in humans). In addition, several model organisms have incomplete snoRNA annotations and their rRNA modification maps are not yet available. Additionally, while online platforms such as Ribosome.xyz and Ribovision provide valuable tools for localising the specific rRNA nucleotides subject to snoRNA-guided modifications within the ribosomal subunits, domains, and helices, translating this structural information into functional insights remains a challenge. Firstly, the detailed knowledge of the functions of individual ribosomal domains and helices is relatively scarce. Secondly, much of the foundational ribosome and translation research has been conducted in prokaryotes or yeast, making it difficult to extrapolate findings to other species. This underscores the need for improved cross-species alignment and nomenclature standardisation. Finally, the understanding of how differential rRNA modifications affect ribosomal protein composition, stoichiometry and ultimately function is mostly missing.

Establishing clear nomenclature guidelines, standardised data formats and a unified framework would facilitate cross-study and cross-species comparisons. Building comprehensive, accessible resources, outlining updated standardisation guidelines and integrating insights from structural biology, biochemistry, and functional genomics will be instrumental to lower the entry barrier, encourage broader participation, and further accelerate discoveries in the field of ribosome heterogeneity and functional specialisation.

P19

Alterations of the 2'O-methylation of ribosomal RNA in IDHwt Glioblastomas

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High-grade adult-type diffuse gliomas (HGGs) constitute a heterogeneous group of aggressive tumors that remain mostly incurable. Recent advances highlighting the contribution of ribosomes to cancer development have offered new clinical perspectives. We uncovered that IDHwt and IDHmut HGGs display distinct alterations of rRNA epitranscriptomics, which could constitute a novel hallmark that can be exploited for the management of these pathologies. We analyzed the ribosomal RNA 2'O-ribose methylation (rRNA 2'Ome) using RiboMethSeq and bioinformatics tools that we developed in-house (https://github.com/RibosomeCRCL/ribomethseq-nf, rRMSAnalyzer) to explore large cohort of patient samples. Our analyses were performed on three independent cohorts compiling 71 HGGs (IDHwt n=30, IDHmut n=41) and 9 non-neoplastic samples. Unsupervised analysis demonstrated that HGGs could be distinguished based on their rRNA 2'Ome epitranscriptomic profile, with IDHwt glioblastomas (GB) displaying the most significant alterations of rRNA 2'Ome at specific sites. Building on patient-derived cell lines, we also demonstrated that distinct cell population present constitutive of GB tumors may display specific alterations of 2'Ome. These observations reveal new cancer-specific alterations of the ribosome biology that may represent new vulnerabilities that could be therapeutically targeted.

P20

Addressing ribosome heterogeneity to untangle translational control and tumor aggressiveness in rare and heterogeneous tumors as malignant pleural mesothelioma

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Malignant Pleural Mesothelioma (MPM) is a lethal cancer with no efficient treatment options, caused by asbestos fibers accumulation in the pleural cavity. A hallmark of MPM cells is their ability to survive chronic stressful environment thanks to highly efficient remodeling of basic cellular processes. From our preliminary data and some hints in the literature, we hypothesize that MPM cells strongly rely on translation remodeling as a central node of adaptation to stressful environment both in disease onset and during the dedifferentiation towards aggressive histotypes.

Our fundamental aim is to characterize the evolving landscape of the translatome network and ribosomal heterogeneity throughout the progression and dedifferentiation of MPM, from healthy pleura to the most aggressive histotypes. However, this endeavor is fraught with unique challenges. The rarity of MPM presents a significant obstacle in acquiring sufficient, high-quality patient-derived samples, compounded by inherent tumor heterogeneity and the difficulty in obtaining healthy pleura from highly inflamed environments. Consequently, the intricate landscape of ribosomal heterogeneity and its dynamic impact on translation during MPM tumorigenesis remains critically under-explored, potentially introducing biases in our understanding based on limited data. To address these limitations, the specific application of newly developed ultrasensitive high-throughput techniques, characterized by their low input requirements and capacity to analyze RNA isoforms and modifications, offers a promising solution. The setting of these techniques in patients' samples of different histotypes in a prospective study could allow the creation of a comprehensive database that would be of great importance in the field. Addressing these challenges is paramount to unlocking the pivotal role of ribosomes and translation dynamics in MPM evolution, paving the way for novel therapeutic strategies.

P21

From Flies to Humans: Decoding the function of rRNA Maturation Across Species

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The study of nucleolar proteins involved in ribosomal RNA (rRNA) maturation presents significant challenges in RNA biology, especially when comparing findings across model organisms and linking basic mechanisms to human diseases. Our research focuses on the nucleolar proteins NOC1, NOC2, and NOC3, which regulate rRNA processing. In humans, their homologs exhibit differential expression in various tumors, with implications for cancer progression. These proteins show varying levels of expression across tumor types, and reduced expression correlates with altered rRNA processing and impaired ribosome maturation. This suggests that these proteins act context-dependently, contributing to both tumor suppression and promotion depending on expression levels.

Conserved from yeast to vertebrates, these proteins play crucial roles in ribosome biogenesis and cellular homeostasis. However, their functional roles can vary across species, raising the question of how to meaningfully interpret data from Drosophila in the context of vertebrate RNA biology, particularly in tumor biology and genome stability. In our lab, we aim to uncover regulatory mechanisms conserved across species by investigating RNA modifications, ribosomal protein functions, and the relatively unexplored area of RNA-DNA hybrid formation. We hope to demonstrate the utility of Drosophila in advancing our understanding of molecular mechanisms driving gene regulation, cellular processes, and disease.

Despite its value in RNA biology, Drosophila's potential is often underappreciated, particularly in understanding the complex regulation of ribosome function. One area of interest is understanding the contrasting roles of "Minute" versus non-Minute ribosomal proteins in RNA synthesis regulation. Given its genetic tractability and well-mapped genome, Drosophila remains an ideal system for investigating these questions. While my studies do not directly address the main focus of this meeting, I find the challenge of translating conserved nucleolar protein roles from Drosophila to humans essential in advancing our understanding of RNA biology, particularly in cancer and neurodegenerative diseases, where ribosome dysregulation is critical.