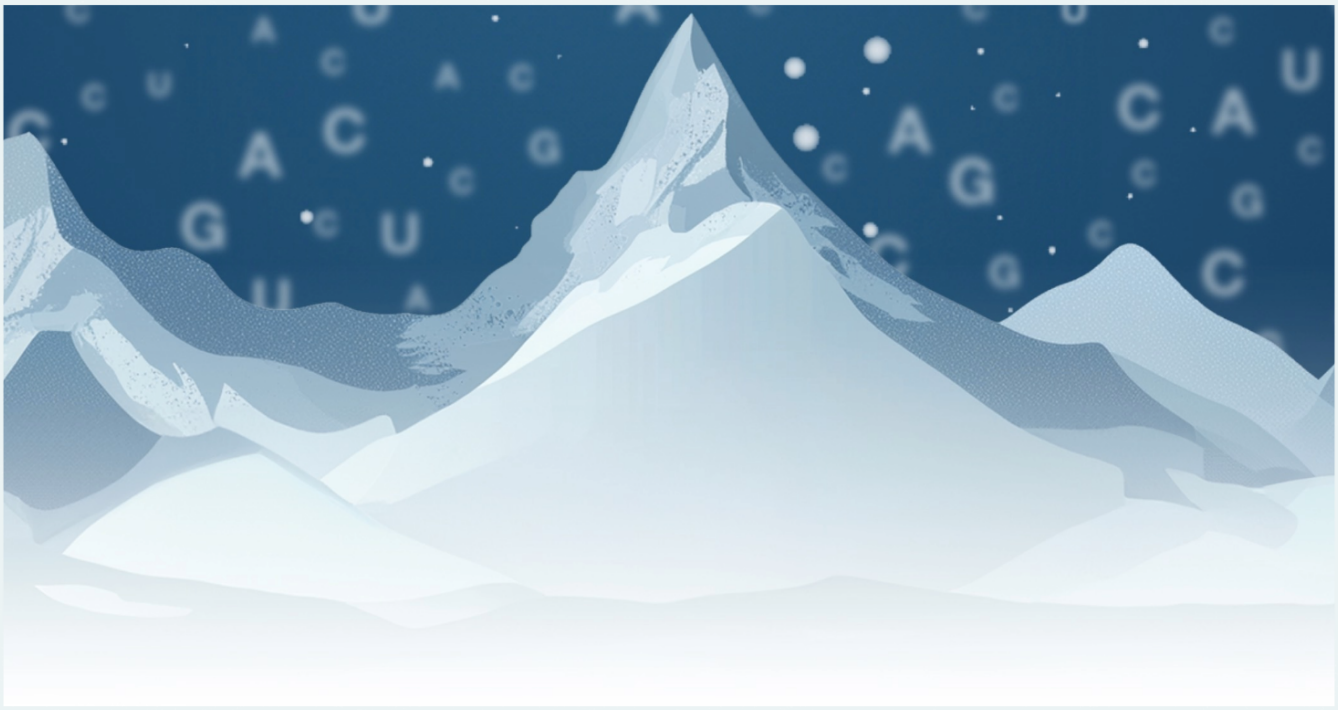
Molecular Mechanisms of RNA in Disease

NCCR RNA & Disease Final Symposium in the Swiss Alps



26 - 29 January 2026

St. Moritz, Switzerland

PRESENTED TO YOU BY

NCCR RNA & Disease, Switzerland

RNAmed, Germany

RNA-DECO & RNA@CORE, Austria

RNA groups from Strasbourg, Montpellier, Bordeaux and Paris, France

OUR FUNDING

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WELCOME MESSAGE FROM THE ORGANIZERS

We are very excited to welcome you all in St. Moritz for the Symposium on “Molecular Mechanisms of RNA in Disease”.

In the context of the final symposium marking the end of our NCCR on RNA and Disease that run since 2014, we will be presenting the most recent scientific achievements from our Swiss network. We are very happy that RNA networks from Germany, Austria and France decided to join us here together with eight fantastic keynotes speakers.

We hope that this will stimulate new collaborations between the labs of different countries in the spirit of what we established within our Swiss-wide network over the last decade.

We wish you all an enjoyable and stimulating meeting.

Frédéric Allain
(Co-director of the NCCR RNA & Disease and Co-Organizer of the Symposium)

Fréderic Allain
ETH Zurich

Norbert Polacek
University of Bern

Sebastian Leidel
University of Bern

Pei-Hsuan Wu
University of Geneva

Michael Jantsch
University of Vienna

Jörg Vogel
University of Würzburg

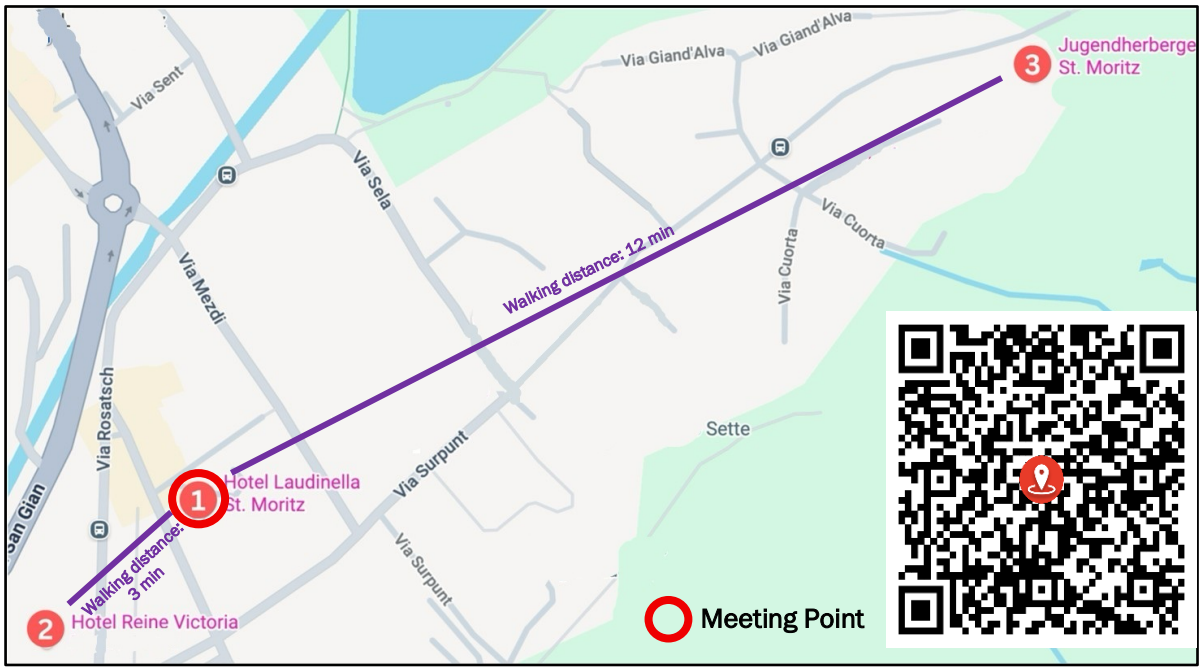
Eric Westhof
University of Strasbourg

GENERAL INFORMATION

Symposium Venue & Accommodation

The symposium takes place in the village of **St. Moritz Bad**, located in the Engadin Valley, a high-alpine region known for its altitude, climate, and mountainous landscape. St. Moritz lies at approximately 1,822 m above sea level. In winter, the valley is typically snow-covered, with temperatures around – 10 °C and plenty of sunshine.

The main symposium venues are **Hotel Laudinella** and **Hotel Reine Victoria**. Participants will be accommodated at Hotel Laudinella, Hotel Reine Victoria, and the St. Moritz Youth Hostel. Detailed accommodation information will be provided by e-mail.



1	**Hotel Laudinella** Via Tegiatscha 17 7500 St. Moritz Website	Seminar Talks (Concert Hall), Poster Session (Bernina Plenary rooms), Lunches & Dinner, Info Desk, Sponsoring Desks, Accommodation **Meeting point on Jan 26th**
2	**Hotel Reine Victoria** Via Rosatsch 18 7500 St. Moritz Website	Poster Session (Theater Hall), Wednesday Night Program (Flying Dinner, Session 9, Party; Theater Hall), Accommodation
3	**Youth Hostel St. Moritz** Via Surpunt 60 7500 St. Moritz Website	Accommodation

St. Moritz Bad is easily walkable, with all venues located close to one another. Please remember to dress warmly and wear appropriate winter footwear.

For detailed directions, please see our **online Google Map** (link or QR code provided on the map).

Getting there by train:

The Engadin Valley is accessible via half-hourly train connections through Landquart or Chur (approx. 2 hours travel time), with St. Moritz as the final stop. The train journey from Chur to St. Moritz passes through a UNESCO World Heritage Site, renowned for its railway engineering integrated into the alpine landscape, including landmarks such as the Landwasser Viaduct.

After arriving at St. Moritz railway station, take bus no. 609 in the direction of *St. Moritz Bad, Youth Hostel*. For Hotel Laudinella and Hotel Reine Victoria, exit at *St. Moritz Bad, Post*; for the Youth Hostel, exit at *St. Moritz Bad, Youth Hostel*.

Visit SBB for public transport schedules and tickets.

Getting there by car:

If you plan to arrive by car from the north, you can either cross the Alps via the Julier Pass or take the RhB car transport shuttle through the Vereina Tunnel (by train, around CHF 39.00 one way). For current road conditions or possible restrictions, please consult the Tiefbauamt (information available in German only). There are a limited number of parking spaces available at the hotel (CHF 15 per night). Please contact the hotel directly for reservations.

Meeting point and registration:

The meeting point is Monday, January 26, 2026, at Hotel Laudinella. Please be in time to register at the Symposium desk, where you will receive your name badge and additional information (between 15:00–16:40).

Start of the meeting: 16:40, Concert Hall, Hotel Laudinella.

Check-In / Check-Out

Check-in: Directly at your hotel upon arrival (from 3:00 pm). Check-out: Thursday morning at the hotel reception (by 10:00 am). Hotel allocation will be provided to you by e-mail prior to the Symposium.

PROGRAM – OVERVIEW

For details on the individual sessions, please refer to page 10-14 of this booklet.

Monday, Jan 26	Tuesday, Jan 27	Wednesday, Jan 28	Thursday, Jan 29
	Breakfast	Breakfast	Breakfast & Check-Out
	08:30-10:00 Session 3: rRNAs & Translation	08:30-10:00 Session 7: RNA quality control and RNA & Genome	08:30-10:00 Session 10: Regulatory ncRNAs and RNA–Protein Complexes
	10:00-10:30 Coffee Break (sponsored by RocketVax)	10:00-10:30 Coffee Break (sponsored by Umlaut.bio)	10:00-10:30 Coffee Break
	10:30-12:00 Session 4: Small RNAs & Viral Infections	10:30-12:15 Session 8: tRNAs & Translation	10:30-12:30 Session 11: Disease Models & Therapies
	12:00-13:30 Lunch	12:15-14:00 Lunch	12:30-14:00 Lunch
	13:30-15:30 Session 5: RNA processing & Disease	Free Afternoon	
15:00-16:40 Welcome Coffee	15:30-16:00 Coffee Break		
16:40-18:30 Opening Remarks & Session 1: RNA Therapeutics	16:00-18:00 Session 6: Genome Engineering & Splicing	17:00-19:00 Poster Session II	
18:30-20:30 Dinner	18:00-20:00 Dinner	19:00-20:30 Flying Dinner	
20:30-22:00 Session 2: RNA Modifications	20:00-22:00 Poster Session I	20:30 Session 9: “Supplementary Materials: Celebrating More Than RNA” & Party	

Meeting Locations

Lobby Hotel Laudinella
Concert Hall (Hotel Laudinella)
Restaurant Stüva (Hotel Laudinella)
Bernina Plenary Rooms (Hotel Laudinella) & Theater Hall (Hotel Reine Victoria)
Theater Hall (Hotel Reine Victoria)

GUIDELINES ORAL AND POSTER PRESENTATIONS

Oral Presentations

The time slots for oral presentations are as follows:

- Keynote Lectures: 25 minutes presentation + 5 minutes Q&A
- Short Talks: 12 minutes presentation + 3 minutes Q&A

Please ensure that your presentation stays within the assigned time slot so the overall schedule can be maintained.

Speakers are kindly asked to bring their presentation either on their own device or as a PDF file on a USB stick during the break preceding the respective session, so it can be set up.

Poster Presentations

Two poster sessions are scheduled, with half of the posters presented in each session:

- **Session I:** Tuesday, January 27, 20:00–22:00 (**odd-numbered posters**)
- **Session II:** Wednesday, January 28, 17:00–19:00 (**even-numbered posters**)

Posters **1–40** will be presented at Hotel Laudinella, in the Bernina Plenary Rooms.

Posters **41–134** will be presented at Hotel Reine Victoria, in the Theater Hall.

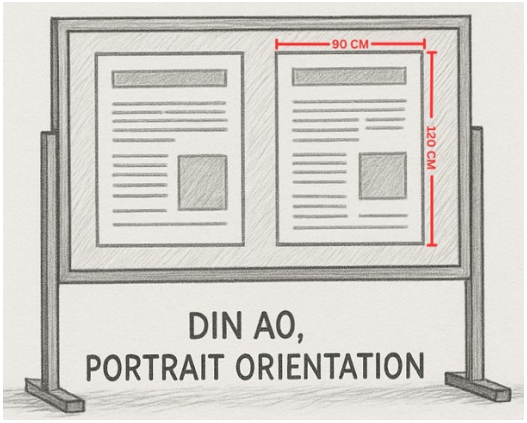
An overview of posters and assigned poster numbers can be found on page 73-76. Please check your poster number, display your poster in the designated room, and present it during the assigned session.

POSTER SETUP AND REMOVAL

Posters may be mounted upon arrival during the Welcome Coffee on Monday afternoon. Please leave all posters on display until Wednesday, after the second poster session, and remove them thereafter.

POSTER DIMENSIONS

Poster boards provide a display area of 90 cm (width) × 120 cm (height). This corresponds approximately to a **DIN A0 poster format** (portrait orientation).



FREE AFTERNOON ACTIVITIES

About St. Moritz & the Engadin Region

St. Moritz is located in the Upper Engadin region of Switzerland. The area is known for its alpine landscape, scenic lakes, and winter climate, and offers a wide range of cultural, recreational, and outdoor activities. For more information, visit the St. Moritz Tourism website: https://www.stmoritz.com/

Below are some suggestions for how you might spend the afternoon on Wednesday. An online **Google Map** with relevant locations is available here: Link

Safety note: Please confirm that your health insurance coverage applies in Switzerland. Check weather conditions (Meteo Schweiz) before outdoor activities and take appropriate care to avoid accidents.

SKIING & SNOWBOARDING

The Signalbahn funicular, located approximately a 6-minute walk from the symposium venue, connects St. Moritz Bad directly with the Corviglia ski area. Enjoy easy access to the slopes and stunning panoramic views of the Engadin mountains: Corviglia Panoramic Map; Ski and snowboard rental is available at Hotel Reine Victoria (Link).

SPA

Hotel Laudinella and Hotel Reine Victoria offer spa facilities with sauna and steam bath. Please contact the hotel reception for information on availability and costs. A day spa is available at Hotel Monopol in St. Moritz, including sauna, steam bath, and Jacuzzi. Bellavita Pool & Spa (Pontresina) provides indoor and outdoor pools and sauna facilities and is reachable in approximately 30 minutes by public transport.

ICE SKATING & NATURAL ICE PATHS

- Ice Arena Ludains (St. Moritz)

Outdoor ice rink within walking distance of Hotel Laudinella. Skate rental available.

- Kulm Olympic Ice Rink St. Moritz

Natural ice rink reachable within 15 min by public transport. Skate rental and lessons available. Check opening times.

- Frozen lake of St. Moritz

Public natural ice rink, official entry next to the Ice Arena Ludains (riding hall in St. Moritz Bad). Skate rental available. Check opening times.

- Ice path in Zuoz

2.5 km route along the Inn River. Rental equipment at Willy Sport Zuoz. About 40–50 minutes from Hotel Laudinella by public transport.

HIKING

- Around Lake St. Moritz

Duration: approx. 1:05 hours | Distance: 4.3km | Difficulty: Easy
A flat loop suitable for all fitness levels, offering views of the lake and surrounding area.

- Lej Nair – Lej Zuppò

Duration: approx. 1:50 hours | Distance: 6.6km | Difficulty: Medium
This route starts in St. Moritz Bad, passes the former Olympic ski jump, and continues through forested terrain to Lej Nair and Lej Zuppò before looping back.

- Chantarella – Salastrains

Duration: approx. 0:50 hours | Distance: 1.4km | Difficulty: Easy
Accessed by funicular from St. Moritz to Chantarella. The return to the valley is via the Signal gondola, which stops near the symposium venue.

- St Moritz - Lej da Staz

45-minute walk (3.8 km) through the forest to the frozen lake Lej da Staz. Option to continue to Pontresina (45 min; return by public transport) or walk back to St. Moritz.

OLYMPIA BOB RUN

Opened in 1904, the Olympia Bob Run St. Moritz is the world's oldest bobsleigh track still in operation and the only one constructed each winter from snow and water. On January 28, it will host training for the Para Sport World Championships. Visitors can access the start and walk alongside the track to the finish (~30 min). Transport: Bus No. 602 or 603 to *St. Moritz, Bären* (~12 minutes); Return: Bus No. 602 or 603 from *Celerina, Cresta Kulm* (near the finish line) back to the seminar location.

PROGRAM OF INDIVIDUAL SESSIONS

Monday, Jan 26, 2026

Arrival & Welcome Coffee

Session 1: RNA Therapeutics (Chair: Frédéric Allain)			
16:40	Allain, Frédéric	*Opening Remarks*	
17:00	Willis, Anne	*Keynote:* Improving the safety profile and enhancing the efficacy of modified in vitro transcribed vaccine RNAs	
17:30	Ryckelynck, Michael	Development of synthetic nucleic acids using ultrahigh-throughput analytical technologies	France
17:45	Rademacker, Stina	Cyclodextrins and lipid nanoparticle formulations for RNA delivery: a good match?	RNA med (Group Merkel)
18:00	Vincent, Mathilde	A role for phosphorothioate stereochemistry in RNA-PROTAC design	NCCR (Group Hall J)
18:15	Campagne, Sébastien	Modular RNA-based PROTACs for selective degradation of multidomain RNA-binding proteins	France

18:30-20:30: Dinner

Session 2: RNA Modifications (Chair: Jörg Vogel)			
20:30	Vaňáčová, Štěpánka	*Keynote*: FTO m^6A RNA demethylase as a safeguard of proper DNA replication in human cells	
21:00	Elhelbawi, Ahmed	The role of N^6-methyladenosine (m^6A) in vertebrate development	NCCR (Group Leidel)
21:15	Jung, Vincent	Toward decoding the combinatorial RNA epigenetic code with RNA Language Models	NCCR (Group Luisier)
21:30	Baharoglu, Zeynep	Epitranscriptomic secrets of bacterial resilience	France
21:45	Helm, Mark	RNA damage from different sources	SAB RNA-DECO

Tuesday, Jan 27, 2026

Session 3: rRNAs & Translation (Chair: Michael Jantsch)			
08:30	Woodson, Sarah	*Keynote*: Chaperoning rRNA folding with helicases, methylases and nucleolar proteins	
09:00	Gleizes, Pierre-Emmanuel	Early alteration of 60S ribosomal subunit assembly in Diamond-Blackfan Anemia Syndrome lead to atypical ribosomal subunits and upper limb developmental defects	France
09:15	Weber, Ramona	Shortcut to protein synthesis: A new paradigm for ribosome loading onto highly translated mRNAs	NCCR (Group Sendoel)
09:30	Mühlemann, Oliver	USP9X coordinates translation repression and ribosome quality control through stabilization of 4EHP and ZNF598	NCCR
09:45	Vosman, Tess	Controlled mRNA translation using 5' cap analogues	RNAmed (Group Rentmeister)

10:00-10:30 Coffee Break

Session 4: Small RNAs & Viral Infections (Chair: Pei-Hsuan Wu)			
10:30	Lohmüller, Michael	Conserved or not? The regulation of let-7 by LIN28	NCCR (Group Grosshans)
10:45	Jouravleva, Karina	Defining the binding properties of each of the four mammalian Argonaute paralogs	France
11:00	Meister, Gunter	RNA meets protein decay – regulation of RNA-guided gene silencing pathways through RNP inactivation and degradation	RNAmed
11:15	Engeroff, Cristina	Small RNA production during genome rearrangement in *Paramecium tetraurelia*	NCCR (Group Nowacki)
11:30	Pfeffer, Sébastien	Contrasting roles of Dicer protein and RNAi during viral infection of bat cells	France
11:45	Lang, Reto	Stress granules formed during different RNA virus infections show remarkable plasticity and substantial virus-specific differences in their formation and composition	NCCR (Group Thiel)

12:00- 13:30 Lunch

Session 5: RNA processing & Disease (Chair: Ulrike Kutay)			
13:30	Gebauer, Fátima	*Keynote*: RNA-binding proteins and translational control in cancer	
14:00	Manglunia, Ruchi	Generating disease-relevant cellular models of TDP-43 pathology	NCCR (Group Polymenidou)
14:15	Lusser, Alexandra	Messenger RNA turnover dynamics is affected by cell differentiation and loss of the cytosine methyltransferase Nsun2	RNA-DECO / RNA@Core
14:30	Sgromo, Annamaria	Kinetic logic of uridylation-mediated RNA decay	RNA-DECO / RNA@Core (Group Ameres)
14:45	Sukyte, Viktorija	Characterization of SKI complex interactors: AVEN and FOCAD	NCCR (Group Jonas)
15:00	Smialek, Maciej	APA-Locate: Alternative Polyadenylation as a spatial code regulator in colon cancer	NCCR (Group Zavolan)
15:15	David, Alexandre	Pioneering cancer therapy through microbiota-derived metabolites and synthetic analogues	France

15:30-16:00 Coffee Break

Session 6: Genome Engineering & Splicing (Chair: Maurice Swanson)			
16:00	Oberli, Seraina	Structural basis of RNA-guided DNA integration by CRISPR-associated transposons	NCCR (Group Jinek)
16:15	Beisel, Chase	Hyper-evolvability at the fringe of CRISPR	RNAmed
16:30	Holzleitner, Noah Ernst	Beyond evolution: Geometric inverse design of non-natural crRNA scaffolds	RNAmed (Group Grünewald)
16:45	Beusch, Irene	Exploring functions of spliceosomal proteins through CRISPR-Cas9 base editing screens	RNAmed
17:00	Trubert, Alexandre	Genome engineering strategies for programmable exon skipping	RNAmed (Group Petri)
17:15	Arora, Rajika	SRSF1 condensates enhance splicing by two mechanisms: RNA protection and surface exposure	NCCR (Group Allain)
17:30	Krainer, Adrian	*Keynote*: Splice-switching ASOs targeting an Alu-Derived Alternative Exon in the *AURKA* 5′ UTR collapse an SRSF1-AURKA-MYC oncogenic circuit in pancreatic cancer	

18:00-20:00 Dinner

20:00-22:00 Poster Session I

Wednesday, Jan 28, 2026

Session 7: RNA quality control and RNA & Genome (Chair: Sebastian Leidel)			
08:30	Caceres, Javier	*Keynote*: RNA-quality control of gene expression in health and disease	
09:00	Baumgartner, Jakob	ChHAP silences SINE retrotransposons by inhibiting TFIIIB recruitment	NCCR (Group Bühler)
09:15	Kutay, Ulrike	Heterochromatin tethering to the nuclear periphery preserves genome homeostasis	NCCR
09:30	Kyriacou, Eftychia	Live cell imaging reveals recruitment mechanisms and dynamics of TERRA R-loops at telomeres	NCCR (Group Lingner)
09:45	Bourgeois, Cyril	MYCN and DEAD-box helicases DDX17 and DDX5 have opposite effects on the production of readthrough-associated chimeric transcripts	France

10:00-10:30 Coffee Break

Session 8: tRNAs & Translation (Chair: Eric Westhof)			
10:30	Tisné, Carine	Structural basis for human mitochondrial tRNA maturation	France
10:45	Glatt, Sebastian	tRNAslational control of eukaryotic gene expression	RNA-DECO / RNA@Core
11:00	Erlacher, Matthias	Which tRNA elements enable tRNA superwobbling?	RNA-DECO / RNA@Core
11:15	Schaefer, Matthias R.	tRNA-derived RNAs: Biogenesis, numbers, molecular interactions and functional potential of a cellular 'waste product'	RNA-DECO / RNA@Core
11:30	Vilardo, Elisa	tRNA modification in development and disease	RNA-DECO / RNA@Core
11:45	Leitner, Moritz	Ashwin and FAM98 paralogs define nuclear and cytoplasmic RNA ligase complexes for tRNA biogenesis	RNA-DECO / RNA@Core (Group Martinez)
12:00	Godin, Juliette	Transfer RNAs: architects of brain development	France

12:15-14:00 Lunch

17:00-19:00 Poster Session II

19:00-20:30 Dinner at Hotel Reine Victoria

20:30-22:00 Session 9: "Supplementary Materials: Celebrating More Than RNA"

22:00 Party

Thursday, Jan 29, 2026

Session 10: Regulatory ncRNAs and RNA–Protein Complexes (Chair: Mary O'Connell)			
08:30	Schneider, Robert	*Keynote*: mRNA binding protein therapeutics for severe muscle injuries, muscle wasting conditions and genetic diseases	
09:00	Suspitsyna, Anastasiia	The Fault in our Vaults: Distinct roles of vault RNA paralogs in liver cancer cells	NCCR (Group Polacek)
09:15	Dueck, Anne	Decoding lncRNA-mediated Regulation of Macrophage Function in Inflammation and Repair	RNAmed (Group Engelhardt)
09:30	Shibata, Keigo	Paternal loss of pi6 piRNAs disrupts the early embryogenesis in mice	NCCR (Group Wu)
09:45	Brehm, Martin	Effector-mediated functional switching of PETISCO between piRNA processing and histone mRNA storage	RNA-DECO / RNA@Core (Group Falk)

10:00-10:30 Coffee Break

Session 11: Disease Models & Therapies (Chair: Norbert Polacek)			
10:30	Fürst, Anna	Establishment of a human iPSC model of inflammatory diseases for drug development	RNAmed (Group Lohse)
10:45	Vesely, Cornelia	Edited Filamin A in myeloid cells reduces intestinal inflammation and protects from colitis	RNA-DECO / RNA@Core (Group Jantsch)
11:00	Brandt, Laura	Extrahepatic delivery of siRNA by nonviral protein cages	NCCR (Group Stoffel)
11:15	Vogel Jörg	ASO technology for phage functional genomics and therapy	RNAmed
11:30	Arpa, Enes Salih	SMG6-dependent nonsense-mediated decay maintains dsRNA homeostasis and suppresses immunogenic transcripts in a hepatocellular carcinoma model	NCCR (Group Gatfield)
11:45	Cooper, Tom	*Keynote*: Mechanistic insights of RNA toxicity and disrupted RNA processing from modeling Myotonic Dystrophy in mice	
12:15	Polacek, Norbert	*Closing Remarks*	

12:30-14:00 Lunch

End of the meeting

TALK ABSTRACTS

Session 1

Improving the safety profile and enhancing the efficacy of modified in vitro transcribed vaccine RNAs

RUST M, SIMMONS-ROSELLO E, SALMON J ALEXANDER FERREIRA, MULRONEY TE, GOH C, THAVENTHIRAN JE and WILLIS AE

University of Cambridge, UK

The elongation stage of protein synthesis represents a major regulatory node of gene expression, and the ribosome has a direct role in "sensing" modified bases in mRNA. For example, codons modified with m^6A are decoded inefficiently by the ribosome, such that they are sensed as "non-optimal" inducing ribosome collisions on cellular transcripts. Other types of RNA modification similarly impact on translation fidelity, and of interest are the effects of modified ribonucleotides incorporated into therapeutic IVTmRNAs to decrease their innate immunity. We showed that incorporation of N1-methylpseudouridine into mRNA caused +1 ribosomal frameshifting *in vitro* and *in vivo.* The +1 ribosome frameshifting is a consequence of N1-methylpseudouridine-induced ribosome stalling during IVTmRNA translation, with frameshifting occurring at slippery sequences. We identified cellular immunity in mouse and humans to +1 frameshifted products from BNT162b2 mRNA translation after vaccination and more recently that frameshifted peptides reduce the immune response. While the COVID vaccine RNAs are safe, there is a theoretical possibility that in future products these could induce off-target immune events. Therefore, we investigated ways in which to ameliorate frameshifting and show that mutations targeting slippery sequences in BNT162b2, using pseudouridine rather than (N)1-methylpseudouridine and the introduction of +1 out-of-frame stop codons can provide effective strategies to reduce the production of frameshifted products and enhance the immune response. Overall, these data increase our understanding of how modified ribonucleotides affect the fidelity of mRNA translation and demonstrate the requirement for further RNA vaccine optimisation.

Session 1

Development of synthetic nucleic acids using ultrahigh-throughput analytical technologies

RYCKELYNCK, Michael

University of Strasbourg, CNRS, IBMC, France

Nucleic acids are powerful polymers for the development of molecular tools with applications in diverse fields, including synthetic biology, diagnostics, and therapeutics. Natural sequences can serve as starting points for such tools, but synthetic molecules can also be rapidly identified in either natural (DNA or RNA) or non-natural chemistries using a variety of methodologies. Aptamers, short oligonucleotides that adopt defined three-dimensional structures enabling them to bind specifically to targets ranging from small ions to large proteins, belong to this class of molecules and are often referred to as “chemical antibodies”. Synthetic aptamers are typically discovered through in vitro selection methods collectively known as SELEX, which make it possible to obtain aptamers with high affinity and binding specificity. However, because selection pressure is usually applied primarily to binding (and sometimes to structure switching, as in Capture-SELEX), these in vitro selection strategies remain limited when it comes to discovering nucleic acids with functions beyond simple binding. This presentation will describe how advances in droplet microfluidics, high-throughput sequencing, and bioinformatics now enable the rapid identification of aptamers optimized either for fluorescence-based detection of small target molecules or for the specific inhibition of target enzymes. In addition, the use of non-natural nucleotide chemistries can further enhance the performance of these molecules in challenging environments.

Session 1

Cyclodextrins and lipid nanoparticle formulations for RNA delivery: a good match?

RADEMACKER, Stina (1), WINKELJANN, Benjamin (1), DUBIELLA, Christian (2), MERKEL, Olivia (1,3,4)

1: Department of Pharmacy, Ludwig-Maximilians-Universität München, Butenandtstrasse 5-13, 81377 Munich, Germany;
2: WACKER Chamie AG, Gisela-Stein-Straße 1,81671 Munich, Germany;
3: Center for NanoScience (CeNS), Ludwig-Maximilians-Universität München, 80799 Munich, Germany;
4: Ludwig-Maximilians-Universität München, Member of the German Center for Lung Research (DZL), Germany

Lipid nanoparticles (LNPs) are of high importance for modern RNA therapeutics and have enabled the clinical success of siRNA- and mRNA-based drugs, including the COVID-19 vaccines [1, 2]. Their performance is governed by the interplay of lipid components that fulfil distinct structural and functional roles within the LNPs. Although highly effective, current formulations still face challenges related to stability and processing, motivating interest in excipients that protect LNPs during drying or storage. Cyclodextrins (CDs) are well-established pharmaceutical excipients characterized by a toroidal architecture with a hydrophobic inner cavity capable of forming inclusion complexes with lipid-like molecules [3]. This property is leveraged in several approved drug formulations and has made β-cyclodextrin derivatives such as hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin (M-β-CD) particularly attractive [4]. Growing evidence indicates that CDs can stabilize lipid-based nanocarriers during drying or freeze–thaw procedures and may also protect nucleic acids in liposomal systems [5-7]. As CDs interact with various lipids, especially cholesterol, it is critical to understand their specific interactions with individual LNP components [8]. Such interactions may offer stabilization benefits but can also induce structural perturbations. In this study, we investigated how HP-β-CD and M-β-CD influence LNPs and observed a clear concentration-dependent effect: higher CD concentrations exerted detrimental effects due to pronounced lipid scavenging. HPLC analysis revealed substantial alterations in LNP lipid composition at elevated CD levels. Additionally, particle size and dispersity, RNA encapsulation, and in vitro performance were assessed to clarify the mechanistic impact of CDs and enable their rational use in future LNP formulations.

[1] Akinc, A., et al., The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat Nanotechnol, 2019. 14(12): p. 1084-1087.
[2] Schoenmaker, L., et al., mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. Int J Pharm, 2021. 601: p. 120586.
[3] Szejtli, J., Introduction and general overview of cyclodextrin chemistry. Chemical reviews, 1998. 98(5): p. 1743-1754.
[4] Kali, G., S. Haddadzadegan, and A. Bernkop-Schnürch, Cyclodextrins and derivatives in drug delivery: New developments, relevant clinical trials, and advanced products. Carbohydrate Polymers, 2024. 324: p. 121500.
[5] Lee, H., D. Jiang, and W.M. Pardridge, Lyoprotectant Optimization for the Freeze-Drying of Receptor-Targeted Trojan Horse Liposomes for Plasmid DNA Delivery. Molecular Pharmaceutics, 2020. 17(6): p. 2165-2174.
[6] van den Hoven, J.M., et al., Cyclodextrin as membrane protectant in spray-drying and freeze-drying of PEGylated liposomes. International Journal of Pharmaceutics, 2012. 438(1): p. 209-216.
[7] Castillo Cruz, B., et al., A Fresh Look at the Potential of Cyclodextrins for Improving the Delivery of siRNA Encapsulated in Liposome Nanocarriers. ACS Omega, 2022. 7(4): p. 3731-3737.
[8] Szente, L. and É. Fenyvesi, Cyclodextrin-Lipid Complexes: Cavity Size Matters. Structural Chemistry, 2017. 28(2): p. 479-492.

Session 1

A role for phosphorothioate stereochemistry in RNA-PROTAC design

VINCENT, Mathilde (1), WELLER, Céline (1), KNECHTLE, Oliver (1), HAAB, Tobias (1), BRUNNER, Cyrill (2), CLERY, Antoine (3), ALLAIN, Frédéric (3), HALL, Jonathan (1)

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Proteolysis-targeting chimeras (PROTACs) are a therapeutic modality that degrade proteins by recruiting the ubiquitin–proteasome system (UPS) [1]. Our lab previously demonstrated that RNA-PROTACs can selectively degrade the cytosolic form of the RNA-binding protein (RBP) Lin28 [2]. RBPs are essential for cellular homeostasis, and dysregulation of TDP-43 and FUS RBPs, is linked to neurodegenerative diseases [3,4].
Targeting RBPs with small molecules is challenging because they lack binding pockets for drug-like molecules. However, their RNA-binding elements (RBEs) can be exploited using short oligoribonucleotides. Since RNA is inherently unstable in vivo, chemical modifications are required. One prominent modification is the phosphorothioate (PS) group, in which a sulfur atom replaces a non-bridging oxygen in the phosphate backbone [5]. PS modifications increase RNA stability and biodistribution but introduce chirality at the phosphorus atom, generating diastereoisomers whose stereochemistry can significantly influence RNA–protein interactions [6,7].
We are developing a method to exploit PS stereochemistry to optimize RNA–protein binding. As a model system, we use RBFOX, a well-characterized RBP with a short consensus RBE (5'-UGCAUGU-3') [8]. Surface plasmon resonance measurements revealed substantial differences, up to 70-fold, in the binding affinities of RBFOX RBE diastereoisomers. We next attached linkers and E3 ligase ligands to the RBFOX RBE and observed reduced RBFOX2 levels in HEK293T cells.
Through this work, we aim to demonstrate that controlling PS stereochemistry can enhance the stability, selectivity, and potency of RNA-PROTACs. We are now applying these findings to RNA-PROTACs that target TDP-43 and FUS in collaboration with the Allain and Polymenidou labs.

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Session 1

Modular RNA-based PROTACs for selective degradation of multidomain RNA-binding proteins

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RNA-binding proteins (RBPs) are central regulators of gene expression, yet their modular and dynamic architectures have hindered efforts to pharmacologically target them. The splicing factor RNA Binding Motif 39 (RBM39) is an attractive anti-cancer target, currently addressed by aryl sulfonamides that act as molecular glues to promote DCAF15-dependent degradation. However, their clinical efficacy is contingent on DCAF15 expression. Here, we report a modular RNA-based PROTAC strategy for DCAF15-independent, selective depletion of RBM39. Guided by the tandem organization of the RBM39 RNA recognition motifs (RRM1 and RRM2), we engineered a high-affinity RNA aptamer that simultaneously engages both domains. Biophysical and structural analyses revealed the relative arrangement of both RRMs required for multivalent binding. Conjugation of this aptamer to the cereblon ligand pomalidomide enabled proteasomal degradation of RBM39 in colorectal cancer cells. The resulting RNA-PROTAC induced dose-dependent RBM39 degradation, impaired splicing function, and suppressed cancer cell proliferation, while unconjugated aptamer or free pomalidomide had only a limited effect. These findings establish RNA-guided PROTACs as programmable tools to selectively degrade multidomain RBPs, extending targeted protein degradation beyond the scope of conventional small molecules and providing a new framework for therapeutic intervention in RBP-driven diseases.

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Session 2

FTO m^{6}A RNA demethylase as a safeguard of proper DNA replication in human cells

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N^6-methyladenosine (m^{6}A) and N^6-2'-O-dimethyladenosine (m^{6}Am) are two highly prevalent and dynamic eukaryotic mRNA modifications that exert regulatory effects on multiple steps of mRNA metabolism. M^{6}A, in particular, has also been shown to regulate a vast majority of different cellular processes, including their newly identified roles in the maintenance of genome stability.
M^{6}A and m^{6}Am can be removed by the activity of the Fat mass and Obesity associated factors (FTO). FTO targets coding and noncoding RNAs. It regulates mRNA processing and stability. We have previously observed that depletion of FTO, an RNA demethylase of m^{6}A and m^{6}Am, leads to increase of marks of damaged chromatin. Furthermore, we observed FTO in the proximity of DNA replication and repair factors. Following up these results, we found out that FTO, is a critical player in replication fork dynamics. Our omics studies reveal physical and genetic interactions between FTO and DNA replication factors. We identify that FTO is physically present at sites of active replication and its demethylation activity is critical for normal replication fork progression. Under conditions of replication stress, FTO exercises a protective role over stalled replication forks to prevent the degradation of nascent DNA. Prolonged replication stress in the absence of FTO was seen to result in DNA strand breaks, possibly due to the collapse of stalled replication forks. Collectively, our results demonstrate a previously unknown role of FTO in the maintenance of replication fork integrity and provide a functional link between RNA modifications and DNA replication.

Session 2

The role of N^6-methyladenosine (m^{6}A) in vertebrate development

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Cellular RNA contains a plethora of chemical modifications that regulate gene expression. N^6-methyladenosine (m^{6}A) is the predominant internal modification in eukaryotic mRNA. It is introduced by a methyltransferase complex (MTC) containing the catalytic subunit METTL3 and two additional constitutive complex members: METTL14 and WTAP. m^{6}A is implicated in diverse biological processes ranging from obesity to cancer. However, the intricate mechanistic roles of m^{6}A in these processes remain unclear. To investigate these mechanisms, we profiled the developmental dynamics of m^{6}A in zebrafish and identified a set of signaling genes that are differentially m^{6}A -modified during development. We then generated a *mettl3*$^{-/-}$ mutant fish which die within a month after fertilization. Bulk and single-cell RNA-seq of Mettl3$^{-/-}$ mutants revealed dysregulation of eye-disease associated genes and underrepresentation of several eye-specific cell types. Histological analysis uncovered significant morphological changes of *mettl3*$^{-/-}$ retinas, while electroretinography disclosed striking visual defects. The mutants also displayed progressively worsening locomotor deficits in dark-light transition assays. Notably, m^{6}A orchestrates eye development by differentially regulating specific eye development genes. Finally, we found that mutant cells respond to the lack of m^{6}A by autoregulating the splicing of wtap. Our work provides a framework for understanding how m6A functions during vertebrate development.

Session 2

Toward decoding the combinatorial RNA epigenetic code with RNA Language Models

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RNA localization, translation, and organelle interactions arise from context-dependent molecular networks. These processes are strongly influenced by RNA post-transcriptional modifications, yet the combinatorial code through which chemical modifications and RNA-binding protein (RBP) interactions specify regulatory outcomes remains elusive. Because experimentally mapping all combinations of RNA modifications, RBPs, and cellular contexts is infeasible, computational approaches are essential for inferring missing data and uncovering underlying principles.
RNA language models (RNA-LMs), trained on millions of unlabelled sequences by predicting hidden nucleotides have shown promise for modeling RNA regulation. However, the biological information they capture at nucleotide resolution remains largely unexplored. Here, we systematically evaluate RNA-LM representations and show that these models learn universal RNA features, while also encoding fundamental biological properties. We find that RNA-LMs operate in two distinct regimes: low-confidence predictions driven by simple sequence properties, and high-confidence predictions informed partly by structural context. Chemically modified sites affect model confidence, for example, m^6A sites have a much lower probability of being predicted as Adenosine compared with randomly sampled positions. We introduce a visualization tool to interrogate internal representations and show that RNA-LMs distinguish m^6A sites, recovering the canonical DRACH motif and additional submotifs. Building on this, we develop a RNA modification predictor achieving state-of-the-art performance and enabling a genome-wide map of predicted modifications, including previously unannotated sites and combinatorial motif patterns.
These results establish RNA-LMs as powerful tools for decoding regulatory logic. Future work will explore the interplay of RNA modifications and RBP binding in RNA metabolism, including localization and translation.

Session 2

Epitranscriptomic secrets of bacterial resilience

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RNA modifications are emerging as key regulators of bacterial physiology, yet their broader roles beyond translation remain poorly defined. In *Vibrio cholerae*, we recently mapped 23 tRNA and rRNA modification enzymes that shape responses to diverse antibiotics. Here, we uncover a striking non-canonical role for one of them, the tRNA dihydrouridine synthase VcDusB.
Beyond its established function in introducing dihydrouridine into tRNAs, VcDusB is essential for oxidative stress resistance. Surprisingly, this protection does not depend on its RNA-modifying activity but instead on an intrinsic NADPH oxidase function that maintains redox balance. Deletion of *dusB* disrupts NADPH homeostasis and renders cells hypersensitive to oxidative damage, while mutational analyses confirm that this redox activity, not dihydrouridylation, is crucial for survival.
Integrating proteomics and transposon insertion sequencing, we show that DusB orchestrates NADPH metabolism and drives adaptive reprogramming under stress. Together, our findings redefine DusB as a bifunctional enzyme that connects RNA modification with redox regulation, revealing an unexpected link between RNA chemistry, metabolism, and stress resilience.
This work broadens the known physiological scope of RNA-modifying enzymes and highlights a new layer of bacterial stress control, where they emerge as central players in cellular defense.

Session 2

RNA damage from different sources

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Compared to RNA modification, RNA damage differs with respect to site and sequence specificity random distribution and stoichiometry. RNA oxidation is an important, though little understood process that has long been overshadowed by research on DNA. A clear shortcoming is the lack of methods to determine the position of oxidized residues in RNA sequences. Their development is hampered by the diversity of oxidation products and their diffuse distribution and low stoichiometry. Here we report the application of a deep sequencing method that capitalizes on aniline-induced strand scission at noncanonical nucleoside structures to generate ligation-competent fragments. Leveraging these as specific molecular entry points for library preparation, the resulting exquisite signal-to-noise ratio allowed the detection of widespread but low amplitude signals at purine sites of yeast RNA, especially at guanosines. As a chemical tool we used hypochlorous acid, a cell-penetrating exogenously added oxidant, and found that treatment of yeast cells boosted signal amplitude, but maintained the general pattern at guanosine residues. Side-by-side quantification of 8-oxoguanosine (oxo^{8}G) and abasic sites before and after oxidation showed that even under physiological conditions, abasic sites were more abundant. Predominantly the latter were enhanced by oxidation, *in vivo* as well as *in vitro*. We show that RNA oxidation at guanosines proceeds via transient oxo^{8}G and results in abasic sites of significant abundance, which constitute the predominant molecular oxidation product that can be site-specifically mapped by our newly developed method termed OAbSeq. We posit that abasic sites are a superior marker for oxidation damage of RNA than oxo^{8}G.

Session 3

Chaperoning rRNA folding with helicases, methylases and nucleolar proteins

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Newly transcribed pre-ribosomal RNA may initially fold into structures that are incompetent for assembly, requiring chaperones to smooth the earliest steps of ribosome synthesis. We investigated the mechanism of rRNA folding and assembly using single-molecule fluorescence microscopy and footprinting. The results show that the *E. coli* DEAD-box protein CsdA accelerates ribosomal protein uS4 binding by unfolding unstable rRNA structures, removing unstable RNPs while allowing native RNPs to continue along the assembly pathway. By contrast, the rRNA methylase RsmB facilitates early folding of the rRNA by transiently stabilizing metastable structures that favor the proper order of ribosomal protein recruitment. Lastly, we compared RNA folding inside and outside Nop1 droplets, an abundant protein in the eukaryotic nucleolus. The results show that Nop1 disrupts RNA tertiary structure but not secondary structure. However, this effect depends only on molecular interactions between Nop1 and the RNA. Our results show how the competition between helicases, modification enzymes and RNA binding proteins shape the folding pathway of the nascent pre-rRNA.

Session 3

Early alteration of 60S ribosomal subunit assembly in Diamond-Blackfan Anemia Syndrome lead to atypical ribosomal subunits and upper limb developmental defects

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The 5.8S rRNA in eukaryotic large ribosomal subunits exists in both long and short forms, differing at their 5′ extremities, though their respective functions remain poorly understood. We recently studied a multigenerational pedigree with a clinical presentation suggestive of Diamond-Blackfan anemia syndrome (DBAS), a congenital disorder typically caused by haploinsufficiency in ribosomal protein genes, but with an unusually high rate of radial (upper limb) anomalies. Genome sequencing identified a heterozygous loss-of-function mutation in RPL17, which encodes a 60S ribosomal subunit protein not previously associated with DBAS.
Remarkably, lymphoblastoid cell lines (LCLs) derived from affected individuals contained 10–20% of 60S subunits harboring an abnormally short 5.8S rRNA variant (5.8SC), a form that is marginal in healthy cells. Ribosome profiling in these cells revealed upregulated expression of ribosomal proteins but reduced translation of mRNAs involved in tissue development and radial patterning, including components of the non-canonical Wnt signaling pathway.
The presence of 5.8SC rRNA in 60S subunits was not attributable to the mere absence of RPL17, consistent with its essential role in 60S subunit synthesis. Drawing on recent cryo-electron microscopy studies of ribosome biogenesis, we hypothesize that the reduced cellular abundance of RPL17 delays its incorporation into maturing pre-60S particles, thereby disrupting the regulation of 5.8S rRNA exonucleolytic maturation.
Notably, severe congenital radial anomalies in other pedigrees were linked to variants in RPL26, which assembles into pre-60S particles at the same stage as RPL17. This suggests a potential link between 5.8S rRNA structure and developmental mechanisms, warranting further investigation.

Fellman F, Saunders C, O'Donohue MF et al. (2024) JCI Insight. 9(17):e172475.

Session 3

Shortcut to protein synthesis: A new paradigm for ribosome loading onto highly translated mRNAs

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The classic model of translation initiation posits that a fully assembled 43S preinitiation complex—including the eIF2-bound ternary complex—assembles prior to loading onto an mRNA. However, this model does not explain how entire groups of highly translated genes, such as those encoding ribosomal proteins, achieve preferential translation despite relying on the same initiation machinery. Here, we uncover a previously unrecognized mode of preinitiation complex loading that preferentially operates on highly translated mRNAs, which we term PRIME-loading. By systematically mapping the 40S scanning landscape during homeostasis, stress, and recovery, we find that under stress, preinitiation complex loading can occur independently of the ternary complex and several canonical initiation factors. This challenges the assumption that mRNA engagement requires a complete preinitiation complex. Instead, we identify a distinct class of mRNA-bound 40S ribosomes that retain inhibitory factors such as eEF2 and PDCD4. Although these ribosomes are initiation-incompetent, they remain able to scan through canonical start codons into coding regions and 3′UTRs. We further show that S6K1 activity, together with sufficient ternary complex availability, is required to release these inhibitory factors and restore efficient initiation at canonical start codons. Using a genome-wide CRISPR screen to identify the PRIME-loading machinery, we unveil that mTOR-regulated proteins such as LARP1/1B and SERBP1 drive the selective loading onto TOP mRNAs, thereby providing a direct explanation for their preferential translation. Collectively, our findings establish PRIME-loading as a previously unrecognized mechanistic basis for selective, mTOR-dependent translation of highly translated mRNAs under homeostasis and for remodeling of the translational landscape upon ternary complex depletion.

Session 3

USP9X coordinates translation repression and ribosome quality control through stabilization of 4EHP and ZNF598

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Various ribosome-associated quality control pathways monitor translation fidelity, detect and resolve aberrant translation events. Recently, a pathway sensing ribosomes with a blocked A site has been characterized. Small molecules such as NVS1.1 and Ternatin-4 induce ribosome stalling by trapping eRF1 and eEF1A1, respectively, in the ribosomal A site, leading to their ubiquitination and proteasomal degradation [1,2]. In addition to previously identified factors GCN1, RNF14, and RNF25, here we report that the deubiquitinase USP9X is required for efficient proteasomal clearance of eRF1 in NVS1.1-treated cells. While ribosome collisions and the ubiquitination of trapped factors still occur in the absence of USP9X, their proteasomal degradation is delayed. Our results show that USP9X stabilizes 4EHP, which is required to efficiently inhibit translation initiation, and ZNF598, a factor required for resolving collided ribosomes. Inactivation of the deubiquitinase activity of USP9X causes reduced levels of 4EHP and ZNF598, resulting in inefficient translation initiation shutdown and proteasomal degradation of trapped eRF1, respectively. Collectively, this explains the slower clearance of eRF1 from NVS1.1-treated cells that fail to express active USP9X.

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Session 3

Controlled mRNA translation using 5' cap analogues

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mRNA is an emerging medical modality, however, approaches to control its activity lack behind other biologics. We developed an approach to control mRNA translation using 5′ cap analogues that block the binding of the eukaryotic translation initiation factor 4E (eIF4E). In cells, translation of an ectopic mRNA with these 5′ cap analogues is blocked until the 5′ cap modification is removed. These caps are compatible with in vitro transcription and facilitate HPLC-based purification of the resulting capped mRNA, circumventing the need to digest uncapped mRNA produced in the process. We show that these mRNAs are translationally muted in cells, and can be activated for translation by different triggers, such as light or a small molecule, depending on the modification on the cap.

Session 4

Conserved or not? The regulation of let-7 by LIN28

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The RNA-binding protein LIN28 is a phylogenetically conserved regulator of stem cell fates in animals. In both *C. elegans* and mammals, it prevents cell differentiation by repressing the maturation of the *let-7* microRNA (miRNA). In mammals, a LIN28 binding motif (LIN28BM) located in the apical loop of the *let-7* hairpin enables LIN28 to bind either the *pri-let-7* in the nucleus or the *pre-let-7* in the cytoplasm. Nuclear binding blocks the initial processing step, while cytoplasmic binding recruits TUT4 terminal uridyl transferase that uridylates the 3' end of *pre-let-7*, triggering its degradation.
Although the mature *let-7* is identical in humans and *C. elegans*, the LIN28-BM is absent in the apical loop of the hairpin in *C. elegans*, suggesting an alternative mechanism of LIN28-mediated *let-7* repression. Indeed, we find that animals with a complete *pup-2/TUT4* knock-out show wild-type *let-7* levels. Transcriptomic analysis supports regulation at the level of *pri-let-7*, which we confirm by smFISH-based analysis. Specifically, spatial transcriptomics and RNA-seq point towards a mechanism involving regulated trans-splicing of the pri-miRNA. Conservation analysis of the *pri-let-7* suggests that the trans-splicing-based mechanism is conserved among nematodes, and we are currently testing this notion for *C. briggsae*. Ongoing mutagenesis of the *pri-let-7* RNA aims to identify RNA regions critical to this novel repression mechanism and to understand its mode of action better. Taken together, our results indicate that despite the striking conservation of biological function and wiring of the *let-7*:LIN28 module, the underlying molecular mechanisms of regulation differ greatly.

Session 4

Defining the binding properties of each of the four mammalian Argonaute paralogs

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In eukaryotes, Argonaute (AGO) proteins loaded with ~21–23 nt RNA guides form the RNA-induced silencing complex (RISC). Mammalian AGO proteins consist of four Argonaute paralogs (AGO1–4), which are expressed in different proportions across various cell types. MicroRNAs (miRNAs) tether AGO1–4 proteins to their targets, triggering mRNA degradation or repressing translation. miRNAs bind targets through their seed sequence—guide bases g2–g7. As few as six base pairs can mediate high affinity interactions, but some RISCs gain considerable binding energy from non-seed nucleotides. Moreover, AGO proteins can function cooperatively over short distances. In addition to this "miRNA" binding mode, extensively complementary sites allow miRNAs to act like siRNAs, directing AGO2 to cleave the target at a single phosphodiester bond across from the center of the miRNA. Recent studies have defined the binding and slicing properties of AGO2, but comparable data is unavailable for AGO1, AGO3 and AGO4, precluding accurate prediction of their targets. We are using RNA Bind-n-Seq and Co-localization Single Molecule Spectroscopy to define the target interactions of the four mammalian Argonaute proteins. Our data should lead to quantitative targeting rules for individual miRNAs bound to AGO1, AGO2, AGO3, or AGO4 and facilitate the development of siRNA, miRNA and antagomir therapeutics with high target specificity.

Session 4

RNA meets protein decay – regulation of RNA-guided gene silencing pathways through RNP inactivation and degradation

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Gene expression is regulated at many post-transcriptional steps of RNA maturation – mediated by non-coding RNAs including microRNAs, lncRNAs or circular RNAs. N^6-methyladenosine (m^6A) on cytoplasmic mRNAs recognized by the reader proteins of the YTH family also regulate gene expression through translation and mRNA degradation. All these pathways play critical roles in diverse cellular and physiological processes and have been associated with cancer development and progression.
Small RNAs including microRNAs and siRNAs bind to Argonaute (AGO) proteins and guide them to complementary target RNAs, which are subsequently degraded. Specific loading mechanisms mediate the processing and transfer of single-stranded mature miRNAs or siRNAs onto AGO proteins. Subsequently, effector proteins such as GW proteins interact with AGO proteins and mediate downstream gene silencing. AGO phosphorylation leads to dissociation from mRNAs and potentially re-entry into gene silencing – a so far hypothetical process that we refer to as 'silencing cycle'. Under specific conditions such as stress or cell cycle arrest for example, AGO proteins become inactivated and the silencing cycle is paused.
When investigating turnover of gene silencing components during such pausing events, we found remarkable similarities between the miRNA and the m^6A pathway, that involve protein phosphorylation, proteasomal degradation as well as autophagy.

Session 4

Small RNA production during genome rearrangement in *Paramecium tetraurelia*

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The ciliate *Paramecium tetraurelia* experiences a highly complex genome rearrangement process during its sexual development. This process includes the elimination of approximately 45,000 transposon-like sequences, which are referred to as Internal Eliminated Sequences (IESs).
The removal of IESs is orchestrated by two distinct classes of small RNAs: scanRNAs (scnRNAs) and iesRNAs generated by three Dicer-like enzymes: Dcl2, Dcl3, and Dcl5 [1,2]. ScnRNAs are characterized by their length of 25 nucleotides and the presence of a 5' UNG signature. The enzyme Dcl2 is responsible for determining the length of these RNAs, while Dcl3 specifies their 5' signature [3]. The production of mature scnRNAs is a sequential process, where Dcl2 and Dcl3 act in succession to cleave and generate the final RNA molecules. IesRNAs, in contrast, are produced by Dcl5 and display variable lengths. They are marked by a 5' UAG signature and a 3' CNAUN signature [2,3]. These small RNAs are derived from excised, concatenated IESs [4]. However, the precise mechanism underlying their production is not yet fully understood.

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Session 4

Contrasting roles of Dicer protein and RNAi during viral infection of bat cells

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Bats serve as reservoirs for numerous viruses that can trigger epidemics in humans and animals, making it essential to understand their antiviral immune mechanisms. Among immune regulators, the conserved ribonuclease Dicer plays a variety of functions in antiviral defense, including processing double-stranded viral RNA through RNA interference (RNAi), generating microRNAs, and indirectly modulating innate immune responses in mammals. To explore Dicer's function in bats, we examined its antiviral activity in *Myotis myotis* nasal epithelial cells. Contrary to expectations, we found little evidence of canonical RNAi activity. Instead, Dicer appeared to exert a mild proviral effect on two alphaviruses, Sindbis and Semliki Forest virus. Notably, Dicer relocalized to distinct cytoplasmic foci during infection. Those structures contained double-stranded and viral positive-strand RNA, suggesting an association with viral replication sites. This relocalization was absent in several human cell lines tested. In contrast, lung epithelial cells from another bat species, *Tadarida brasiliensis*, which was previously shown to exhibit enhanced RNAi activity, showed no Dicer relocalization, indicating that subcellular localization may correlate with antiviral function. Moreover, Dicer relocalization required *M. myotis*-specific cellular factors, and *M. myotis* Dicer displayed antiviral activity when expressed in human cells. These findings suggest that Dicer's antiviral role in bats is context-dependent, varying across species and cell types, and may be determined by its intracellular distribution.

Session 4

Stress granules formed during different RNA virus infections show remarkable plasticity and substantial virus-specific differences in their formation and composition

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Stress granules (SGs) are membraneless accumulations of ribonucleoproteins which form in the cytosol of eukaryotic cells upon exposure to various stress stimuli. These granules are proposed to store untranslated mRNA during stress and support the dynamic reprogramming of translation towards stress resolving pathways. Growing evidence of many viruses specifically interfering with SG formation, and the identification of antiviral sensor and effector proteins in SGs suggests an involvement of these condensates in the antiviral host response. However, the characteristics of virus-induced SGs are poorly understood.
By focusing on SGs induced by the coronavirus mouse hepatitis virus (MHV) and the alphavirus Semliki Forest virus (SFV), we directly compared different aspects of SG formation between two different RNA viruses. An initial comparison of SG formation kinetics by live-cell imaging revealed distinct time points of SG induction between both viruses. By applying APEX2-mediated proximity labelling and quantitative proteomics, we comprehensively compared the SG protein composition between SFV and MHV at different time points of infection revealing substantial virus-specific differences. A further subcellular localization of SG components by microscopy not only confirmed a reduced abundance of several translation initiation factors in MHV-induced granules but also showed large differences in the accumulation of viral RNA between both virus-induced condensates.
While SFV-induced SGs show canonical SG characteristics, MHV-induces an atypical SG type with reduced connection to canonical SG themes. Overall, our findings revealed virus-induced SGs to be strongly virus specific highlighting a large plasticity of these condensates and further indicate different roles of these condensates during infection.

Session 5

RNA-binding proteins and translational control in cancer

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RNA-binding proteins (RBPs) are gaining great attention in the oncology field for their potential to regulate essentially every hallmark of tumor development. Among other processes, RBPs are involved in translational reprogramming of cancer cells, promoting adaptive mechanisms of stress resistance and cell plasticity, ultimately leading to cancer aggressiveness. During my talk I will focus on RBPs involved in melanoma metastasis, with a particular emphasis on CSDE1. I will explain our efforts to untangle its mechanisms of action and evaluate its potential as clinical biomarker and therapeutic target.

Session 5

Generating disease-relevant cellular models of TDP-43 pathology

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are disorders of the nervous system, leading to death due to respiratory failure or paralysis. TDP-43, an RNA-binding protein containing an unstructured low complexity carboxy-terminal domain (LCD), is found aggregated in most ALS and FTD cases. These aggregates, are often found phosphorylated in patient brain tissue, making it an essential diagnostic marker of the pathology. However, it is unclear if phosphorylation is a cause or effect of the aggregation. It is also not known if there exists a mechanistic connection between the loss of normal RNA-binding function of TDP-43 and the gain of toxicity of the aggregates, in the disease progression. We developed a cellular model to study the uptake of patient-derived and recombinant TDP-43 LCD aggregates. We demonstrated that both aggregate types self-propagate in neuron-like cells, eventually mimicking FTD pathology. We assesed the loss of TDP-43 RNA splicing function in seeded cells, using a fluorescent reporter of its function. We observed that the uptake of both types of aggregates caused the recruitment of endogenous TDP-43 leading to loss of nuclear TDP-43, to different extents, and eventually causing loss of function in seeded cells. RNA-sequencing also showed the expression of cryptic exons which are a signature of TDP-43 pathology in FTD patients. We also phosphorylated our recombinant aggregates and confirmed their lack of recruitment capabilities in cells and hence the inability to cause a loss of TDP-43 function. In conclusion, our data highlight mechanistic insights to the pathological phenotypes in ALS/FTD.

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Session 5

Messenger RNA turnover dynamics is affected by cell differentiation and loss of the cytosine methyltransferase Nsun2

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Nsun2 installs 5-methylcytosine (m^5C) on multiple RNA classes, including mRNAs, tRNAs, and other noncoding RNAs. In mRNA, m^5C has been linked to transcript stability, though whether it stabilizes or destabilizes transcripts remains unresolved. To clarify Nsun2's role in mRNA stability, we profiled mRNA turnover during embryonic stem cell (ESC) differentiation in wild-type and Nsun2-deficient cells. Using RNA metabolic labeling paired with thiouridine-to-cytidine sequencing (TUC-seq), we found widespread shifts in mRNA synthesis and decay during normal differentiation. Strikingly, many of these changes did not alter steady-state mRNA levels, revealing strong transcript buffering in the course of differentiation. Loss of Nsun2 similarly reshaped mRNA turnover without changing overall mRNA abundance. Our data further suggest that the impact of Nsun2 on mRNA turnover is not driven by m^5C in mRNA, consistent with catalysis-independent effects of Nsun2 on translation early in ESC differentiation. Altogether, the results show that both differentiation and Nsun2 loss modulate mRNA turnover in ways that are independent of mRNA methylation yet consistent with buffering mechanisms that maintain constant RNA levels.

Session 5

Kinetic logic of uridylation-mediated RNA decay

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Non-templated nucleotide addition to RNA 3′-ends is a conserved eukaryotic modification with diverse functions, most prominently the targeted degradation of structured RNAs. In Drosophila melanogaster, uridylation-mediated decay is executed by the cytoplasmic Terminal RNA Uridylation-Mediated Processing (TRUMP) complex, which eliminates aberrant RNA polymerase III transcripts, structured introns, and small RNA–bound cleavage products. TRUMP comprises the terminal uridylyltransferase Tailor, which marks substrates, and the exonuclease Dis3L2, which degrades them via a uracil-specific active-site tunnel. How Tailor primes substrates for efficient Dis3L2-mediated decay remains unclear.
Using high-throughput biochemical assays and kinetic modeling, we dissected uridylation-coupled RNA decay. Tailor's substrate selectivity depends on the 3′ terminal nucleotide but is overridden by base pairing, ensuring robust uridylation of structured RNAs. Tailor initiates distributive uridylation and transitions to a processive mode, a switch attenuated by sporadic A/C incorporation due to relaxed NTP selectivity. Oligouridylation generates stable intermediates of three to five U's, which optimally license Dis3L2 substrate engagement. Massively parallel measurements of decay kinetics and high-throughput affinity profiling revealed that Dis3L2 productively engages structured RNAs only when they contain a ≥4-nucleotide 3′-overhang. Efficient decay requires balancing high-affinity binding with threading along the channel to the active site, a regime optimally fulfilled by tailing products. Notably, tolerance for non-U nucleotides is restricted to 3′-most position, indicating that promiscuous A/C incorporation by TUTases does not compromise Dis3L2-directed decay.
Our findings define the kinetic principles coupling Tailor-dependent uridylation to Dis3L2-mediated degradation within TRUMP, uncovering the fundamental mechanism by which RNA 3′ uridylation generates decay-competent substrates and enforces cytoplasmic RNA surveillance.

Session 5

Characterization of SKI complex interactors: AVEN and FOCAD

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mRNA decay plays a vital role in preserving healthy transcriptome levels. Protein coding transcripts that do not pass integrity checks are quickly degraded via quality control pathways closely connected to translation in mammals. mRNA fragments generated during these mRNA surveillance pathways are degraded from their 3' ends via the RNA exosome. In the cytoplasm, the key RNA exosome cofactor is the SKI-SKIC7 complex, which has been recently shown to interact with two proteins in mammals, AVEN and FOCAD [1]. Both proteins are poorly characterized to date and the molecular basis of their function in cytoplasmic mRNA decay is unclear. Here, we show that AVEN bridges FOCAD, the SKI complex and ribosomes via short binding motifs. Our structural analyses demonstrate that FOCAD acts as a dimeric scaffold for AVEN, and that AVEN and SKIC7 can simultaneously bind the SKI complex. Together, AVEN, FOCAD and SKIC7 increase affinity of SKI complex to ribosomes and enhance the RNA helicase activity of SKIC2. Furthermore, disruption of the AVEN-FOCAD-SKI complex leads to inefficient functioning of mRNA surveillance pathways, NMD and NGD, as well as elimination of histone mRNAs. Collectively, our results suggest that AVEN and FOCAD are SKI complex cofactors that are important for activation of RNA exosome mediated mRNA degradation in the cytoplasm.

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Session 5

APA-Locate: Alternative Polyadenylation as a spatial code regulator in colon cancer

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Alternative polyadenylation (APA) generates 3′UTR isoforms that govern mRNA stability, localization, and translation. The Cleavage Factor Im (CFIm; CPSF5/NUDT21, CPSF6, CPSF7) complex promotes distal PAS usage, yet its role in establishing spatial gene-expression patterns in cancer is unclear. We performed siTOOLs-mediated knockdown of CPSF5, CPSF6, and CPSF7 in HCT116 colorectal cancer cells. RNA-seq revealed widespread 3′UTR shortening upon CPSF5/6 depletion (2,184 and 2,095 genes, respectively), with minimal transcript-abundance changes, indicating that length and abundance are largely orthogonal.

To connect APA to subcellular routing, we combined CFIm perturbation with two purpose-built platforms. (i) Antibody-guided FACS of endogenous organelles/condensates using directly conjugated primary antibodies resolved ≥12 compartments (nucleus, free cytosol, ER, TIS granules, cytoskeleton, mitochondria, endosomes, membrane, Golgi, peroxisomes, lysosomes, P-bodies). ImageStream 60×, RT-qPCR sentinels, and LC-MS/MS set purity thresholds, while low-input A-Seq2–derived 3′-end libraries preserved PAS resolution to build isoform-resolved localization maps ± CFIm knockdown. (ii) Sequential smFISH quantified CD47-all, CD47-long, BAG3, ALDH18A1, and MALAT1 across nucleus, cytosol, ER, and TIS granules.

Among shortened transcripts, CD47 showed enhanced mRNA stability and striking protein mislocalization—from the plasma membrane toward ER/cytosolic compartments—after CPSF5/6 depletion or selective loss of the long 3′UTR isoform, and p-ERK condensates concomitantly dissolved. Known colon spatially resolved mRNA - NET1 emerged as an additional polarity-linked APA target.

These data position CFIm-controlled APA as a spatial code that dictates where RNAs and proteins reside, with consequences for signaling and epithelial architecture. The framework enables causal testing and suggests therapeutic avenues to restore long-UTR routing information in colorectal cancer.

Session 5

Pioneering cancer therapy through microbiota-derived metabolites and synthetic analogues

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Microbial metabolites interact with eukaryotic hosts, influencing cell physiology through various mechanisms. A notable molecular pathway involves the incorporation of the gut microbial metabolite queuine into the wobble position of host tRNAs (as a queuosine nucleotide) by the host enzyme eTGT, thereby regulating host cell translation. Microbes also produce pre-queuosine1 (preQ1), an intermediate in the complex queuosine biosynthesis pathway. We discovered that both preQ1 and queuine are detectable in the plasma and tissues of mice and are incorporated into host tRNAs both in vitro and in vivo.
However, the impact of preQ1 on host cell biology is markedly different from that of queuine. Our recent study demonstrates that preQ1 incorporation into tRNA disrupts protein synthesis, significantly alters gene expression, and inhibits the proliferation of human and mouse cancer cell lines, while having no effect on non-cancerous fibroblast cell lines. Additionally, preQ1 treatment reduces tumor growth in a xenografted cancer mouse model without affecting healthy tissues.
These preliminary observations led us to evaluate the potential of exploiting preQ1 to treat advanced, aggressive cancers. In vitro experiments on several cell lines show that preQ1 has a broad ability to inhibit cancer cell proliferation without apparent toxicity, with an enhanced effect in aggressive cancer cell lines. However, the effect of preQ1 is limited by its competition with queuine for eTGT-mediated RNA incorporation. To address this limitation, we designed a panel of synthetic queuine derivatives and identified one which exhibits a similar inhibitory effect on cancer cell lines but with a linearly increasing competitive efficiency.

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doi: 10.1038/s41556-025-01750-6. Epub 2025 Sep 16.

Session 6

Structural basis of RNA-guided DNA integration by CRISPR-associated transposons

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CRISPR-associated transposons (CASTs) are natural RNA-guided integration systems in which nuclease-deficient CRISPR-Cas machineries have been co-opted by Tn7-like transposons to mediate site-specific DNA insertion [1-3]. By coupling RNA-guided target recognition with transposase-driven DNA integration, CASTs overcome a major limitation of canonical CRISPR genome-editing tools: the inability to programmably insert large genetic payloads without introducing double-strand DNA breaks. Understanding the mechanisms of CRISPR-associated transposons is therefore essential for harnessing these systems as programmable gene insertion technologies. Of particular interest is the type I-F *Pseudoalteromonas sp.* S983 CAST (*Pse*CAST), which has been the focus of extensive engineering efforts and has shown encouraging integration activity in mammalian cells [4,5]. Yet, mechanistic insights into *Pse*CAST remain incomplete, and existing structural information is limited to isolated sub-complexes [6].
To address these gaps, we have combined single-particle cryo-EM with biochemical analyses to capture intermediate and post-transposition states that elucidate the molecular underpinnings of RNA-guided DNA integration in type I-F *Pse*CAST. We resolve the Cascade-TniQ-TnsC transposon recruitment complex, highlighting the pivotal role of the ATPase TnsC in bridging the CRISPR-Cas and transposon modules. Additional cryo-EM structures reveal how the RNA-guided targeting machinery recruits the donor DNA-bound transposase through a series of newly identified protein-DNA and protein-protein interactions. Finally, we describe large-scale domain rearrangements and catalytic site remodeling within the transposase subunit TnsB, indicating an allosteric mechanism that drives transposase activation for DNA integration. Collectively, these findings define the coordinated interplay among the modular components of *Pse*CAST and provide a structural basis for advancing the system as a programmable genome-engineering tool.

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Session 6

Hyper-evolvability at the fringe of CRISPR

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CRISPR is synonymous with powerful tools for gene editing, yet its origins lie in RNA-guided adaptive immune systems present in bacteria and archaea. The associated RNA-guided nucleases enact the system's immune response upon recognition of an invader's nucleic acid sequence and have been coopted as the centerpiece of these gene-editing tools. While Cas9 remains the emblematic CRISPR nuclease, prokaryotes encode a wide diversity of these nucleases with ranging biochemical properties and immune functions that each unlock different types of technologies. In this talk, I will describe my group's work on a small sliver of these nucleases encoding incredible functional diversity that is enabling a wide set of biomolecular tools. This sliver includes Cas12a nucleases known to cut target DNA similar to Cas9, yet the hidden diversity around Cas12a encodes additional properties such as recognizing target RNA and unleashing cleavage of double-stranded DNA or the tails of tRNAs. This diversity in turn is unlocking a collection of new applications, from transcript-dependent DNA shredding that can eliminate human cells based on their transcriptional profile to expanding the scope of multiplexed molecular diagnostics. These insights suggest that much more is to be gained by exploring the fringe of bacterial defenses and their technological potential.

Session 6

Beyond evolution: Geometric inverse design of non-natural crRNA scaffolds

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Natural CRISPR–Cas systems use a narrow set of crRNA scaffold architectures, potentially using only a small subset of the possible sequence-structure space compatible with nuclease function. Although “inverse design” of proteins has advanced rapidly [1], equivalent 3D, tertiary-structure–aware design tools for RNA remain underdeveloped. Here, we use the geometric inverse design platform gRNAde to generate a library of non-natural crRNA scaffold architectures and evaluate their ability to support Casλ activity [2,3]. AlphaFold3 structural predictions of RNA–protein complexes were used to prioritize candidates [4]. Testing in HEK293T cells revealed that several designed scaffolds exhibited comparable or higher activity than the wild-type scaffold. Notably, some functional scaffolds occupy sequence space with little resemblance to known biological Casλ crRNAs. Our results demonstrate that 3D geometry–driven inverse design can be applied to RNA to create functional crRNA scaffolds, complementing language-model–based and sequence-level approaches.

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Session 6

Exploring functions of spliceosomal proteins through CRISPR-Cas9 base editing screens

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Eukaryotic gene expression relies on RNA splicing by the spliceosome. Most spliceosomal proteins are essential for cell survival, limiting what can be learnt from their knockout or knockdown approaches. To identify informative alleles, we implemented CRISPR-Cas9 base editing in haploid human cells (eHAPs), producing a library that generates point mutations in thousands of individual residues across >150 spliceosomal proteins. We tested this approach by screening for mutants resistant or hypersensitive to pladienolide B (plaB), a splicing inhibitor that binds U2 snRNP and prevents A complex formation. Hypersensitive mutants occurred in components of A complex but also in factors acting later in splicing. Resistant mutations showed anticipated sequence changes in proteins near the plaB binding pocket.
Viable resistance mutations map not only to the plaB binding site but also to SF1, a factor displaced by U2 snRNP during spliceosome assembly, and to the G-patch domain of SUGP1. We used these mutants and biochemical approaches to identify the spliceosomal disassemblase DHX15/hPrp43 as the ATPase ligand for SUGP1. These and other data support a model in which SUGP1 promotes splicing fidelity by triggering early spliceosome disassembly in response to kinetic blocks.
The ability to identify functional significant alleles of human spliceosomal proteins in vivo opens up exciting avenues to the study of their functions in a manner not previously possible. Our approach also provides a template for the analysis of other essential cellular machines.

Session 6

Genome engineering strategies for programmable exon skipping

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The investigation of individual exon contributions to T-cell function presents a significant obstacle in T-cell research. However, using exon-level genome editing may provide a valuable understanding to the roles that individual exon plays in T-cell function in a manner where exon functions can be assessed by engineering variants in which that exon is selectively removed. Therefore, we are establishing two different methods for inducing exon skipping at the RNA level using CRISPR-Cas9 genome editing to provide a means of determining the role of specific exons in the immune system. The first is a Dual sgRNA CRISPR/Cas9 system to induce exon skipping, in which sgRNAs are directed to two separate sites, an upstream site and a downstream site surrounding the exon in the genomic region of interest, allowing the targeted deletion of that exon by cleavage and repair by the Cas9 protein [1]. The second approach comprises the application of Cytosine and Adenine Base Editors at the splice site motifs (donor and acceptor sites) following the SPLICER toolbox approach to promote exon skipping without creating double-strand breaks [2]. These complementary methodologies provide us with a way to generate exon-deleted or exon-skipped variants systematically so that we can analyse the functional importance of each individual exon. In summary, dual-Cas9 deletion and splice-site base editing together enable versatile exon-level genome engineering.

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Session 6

SRSF1 condensates enhance splicing by two mechanisms: RNA protection and surface exposure

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Biomolecular condensates have emerged as central regulators of numerous molecular functions. Through a mechanism known as liquid-liquid phase separation, proteins containing low-complexity domains, such as splicing factors, are prone to formation of condensates. Although these condensates are biophysically well characterized, their implication in pre-mRNA splicing remains unclear1-3. Here, we uncover a novel mechanism explaining how condensates enhance splicing. We show that serine-arginine-rich splicing factor 1 (SRSF1) and hnRNPA1 coexist in condensates, and addition of RNA results in their spatial segregation. Phosphorylation of SRSF1 RS domain, which is critical for its splicing activity4, induces RNA to co-localize with hnRNPA1. Addition of a mimic of U1snRNP pulls the 5' splice site (ss) to the interface between hnRNPA1 and SRSF1 droplet, showing that condensates of splicing regulators modulate spatial organization of ss, providing a possible explanation to why spliceosome can dispense with scanning pre-mRNAs. Functional assays reveal that the phosphorylation of SRSF1 regulates the localization of the pre-mRNA within condensates and ultimately modulates the splicing outcome. Under low phosphorylation state, SRSF1 can protect RNA by sequestering it within the core, resulting in low splicing efficiency. In contrast, under higher phosphorylation, both SRSF1 and pre-mRNA relocate to the condensate surface, leading to higher splicing efficiency. We propose that SRSF1 protects RNA by sequestering it within the condensed phase and upon SRSF1 phosphorylation, RNA relocalizes to the droplet periphery, promoting its interaction with the splicing machinery. Historically viewed as passive storage sites, splicing factor containing condensates portray a novel and active role in splicing regulation.

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Session 6

Splice-switching ASOs targeting an Alu-Derived Alternative Exon in the *AURKA* 5′ UTR collapse an SRSF1-AURKA-MYC oncogenic circuit in pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy, driven by oncogenic *KRAS* mutations and dysregulated oncogenes, including *SRSF1*, *MYC*, and Aurora kinase A (*AURKA*). Although KRAS-targeted therapies are in development, resistance mechanisms underscore the need to identify alternative vulnerabilities. We have uncovered an SRSF1-AURKA-MYC oncogenic circuit, wherein SRSF1 regulates *AURKA* 5′ UTR alternative splicing, enhancing AURKA protein expression; AURKA positively regulates SRSF1 and MYC post-translationally, independently of its kinase activity; and MYC transcriptionally upregulates both *SRSF1* and *AURKA*. Elevated SRSF1 in tumor cells promotes inclusion of an Alu-derived alternative exon in the *AURKA* 5′ UTR, resulting in splicing-dependent mRNA accumulation and exon-junction-complex deposition. Modulating 5′ UTR alternative splicing with exon-skipping antisense oligonucleotides (ASOs) collapses the oncogenic circuit, reducing PDAC cell viability and triggering apoptosis. Our findings identify *AURKA* alternative splicing as a critical regulatory node and highlight a potential therapeutic strategy that simultaneously targets *SRSF1*, *AURKA*, and *MYC* oncogenes.

Session 7

RNA-quality control of gene expression in health and disease

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RNA surveillance mechanisms are essential to ensure accurate gene expression [1]. Nonsense-mediated decay (NMD) is a translation-dependent mechanism that degrades mRNAs harboring premature termination codons (PTCs) but also regulates the stability of normal transcripts and plays an important role in multiple physiological processes.
NMD is a translation-dependent mechanism that occurs in the cytoplasm; however, our lab has identified a localized NMD response that regulates the expression of transcripts translated at the Endoplasmic reticulum (ER), termed ER-NMD [2]. We propose that this pathway protects the ER, ensuring quality control of ER-translated mRNAs which, due to their intrinsic localized translation, will not have sufficient exposure to cytoplasmic NMD. I will discuss the NMD pathway, focusing on the functional characterization of ER-NMD.
We have developed a series of transgenic mouse models harboring NMD sensors, which is allowing us to monitor the efficacy, tissue-specificity, and developmental regulation of the NMD response in vivo, with single-cell resolution. We are also using these NMD mouse models to carry out in vivo CRISPR screens that has led to the identification of novel genes involved in NMD regulation in the mouse liver.
We have also developed NMD sensors in osteosarcoma U2OS cells that allowed us to carry out high-throughput screens with small molecule compound libraries with the aim of identifying novel NMD inhibitors. We are using a high-content image-based screening platform to evaluate multiple compound libraries, including FDA-approved drugs, and identified several potential new NMD inhibitors that are currently being validated.

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Session 7

ChHAP silences SINE retrotransposons by inhibiting TFIIIB recruitment

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Short interspersed nuclear elements (SINEs) are abundant non-autonomous transposable elements derived from RNA polymerase III (POL III)-transcribed short non-coding RNAs and constitute a substantial portion of vertebrate genomes. SINEs retain sequence features recognized by the POL III machinery, including the transcription factor complexes TFIIIC and TFIIIB. Despite their impact on genome stability and evolution, the mechanisms governing SINE transcription remain poorly understood. We therefore set out to study their transcriptional regulation in mouse embryonic stem cells (mESCs). Using reverse genetics and genomics approaches, we confirm that SINE B2 elements are transcribed by POL III in mESCs, allowing us to further dissect their regulation. Although DNA methylation and heterochromatin formation have been implicated in SINE B2 repression, we find these pathways play only a minor role in this cell type. Instead, we identify the ChAHP complex, consisting of the Zinc-finger protein ADNP, the heterochromatin binding protein HP1γ and the chromatin remodeler CHD4, as a key repressor of SINE B2 elements. ChAHP directly inhibits POL III transcription by blocking TFIIIB recruitment, without affecting TFIIIC binding. This repression requires the ATPase activity of CHD4, arguing against a model in which ChAHP sterically hinders TFIIIB access. Notably, this repressive activity is selective for SINE B2 elements, as other POL III transcripts, such as tRNAs, remain unaffected by ChAHP loss. Our findings establish ChAHP as a non-canonical repressor of POL III-dependent SINE transcription, offering new insights into the control of this pervasive class of non-coding genomic elements.

Session 7

Heterochromatin tethering to the nuclear periphery preserves genome homeostasis

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Heterochromatin is positioned at the nuclear periphery and around nucleoli, whereas euchromatin resides in the nuclear interior and in vicinity of nuclear pore complexes. This 'conventional' nuclear architecture is an almost universal feature of eukaryotic nuclei and achieved by tethering of heterochromatin to the nuclear envelope (NE). An outstanding question in the field concerns the mechanisms that govern the peripheral positioning of mammalian heterochromatin. Here, we have taken a systematic approach to identify proteins of the INM that promote heterochromatin attachment to the NE in mammalian cells. Using reverse genetics, we show that downregulation of a set of abundant NE proteins causes a global detachment of heterochromatin from the nuclear periphery in different types of cultured human and mouse cells, indicating that heterochromatin anchorage in mammals is accomplished by an evolutionarily conserved mechanism. Long-term loss of the identified tethers leads to changes in 3D genome organization and a reduction of repressive epigenetic marks. These changes are associated with a massive deregulation in gene expression of coding and non-coding RNAs, including the activation of retrotransposons and an activation of antiviral innate immunity. Collectively, this work reveals the impact of genome organization on the orchestration of RNA expression, with downstream consequences on cell fate determination.

Session 7

Live cell imaging reveals recruitment mechanisms and dynamics of TERRA R-loops at telomeres

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Chromosome ends are capped by telomeres, specialized nucleoprotein structures that maintain genomic integrity and regulate cellular lifespan. Telomeres are transcribed into long noncoding RNAs called TERRA. During cellular aging and in ALT cancers, TERRA regulates telomere maintenance by homology-directed repair. These TERRA functions depend on its association with telomeres via R-loops that form post transcriptionally. However, the underlying molecular mechanisms remain only partially understood and the dynamics of its interaction with telomeres unexplored. To characterize the TERRA-telomere interactions in live cells we combine PP7 stem-loops TERRA tagging and genome editing to image single TERRA molecules and telomeres. Using deep-learning tools for spot detection and single particle tracking analysis, we determine telomere and TERRA dynamics in live cancer cells with short or long telomeres. We find that TERRA molecules are highly mobile whereas telomeres are more static. We demonstrate that TERRA visits telomeres via two modes. On one hand, TERRA makes frequent transient interactions with the shelterin protein TRF2. On the other hand, TERRA engages in long-lasting interactions that last from seconds to several minutes, forming R-loop structures with telomeric DNA. The TERRA R-loops occur preferentially at short telomeres, and their formation requires factors involved in homology-directed repair but not TRF2. Altogether, we unravel two parallel mechanisms of TERRA recruitment to telomeres which differ substantially in their dynamics and structure, and they disentangle R-loop dynamics. Our findings provide a framework to understand TERRA functions during aging and carcinogenesis.

Session 7

MYCN and DEAD-box helicases DDX17 and DDX5 have opposite effects on the production of readthrough-associated chimeric transcripts

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In a previous work (Terrone et al., NAR 2022), we have shown that DEAD box helicases DDX17 and DDX5 control the correct termination of transcription and the associated cleavage of the 3' end of transcripts. Accordingly, their knockdown in neuroblastoma cells results in readthrough transcription but also in an increased production of chimeric transcripts (hereafter coined as tracRNAs) from tandemly oriented genes. Exploring the possible mechanisms underlying this phenomenon in neuroblastoma, in which tracRNAs are abundant, we found that a low expression of DDX17 and DDX5 genes is significantly associated with poor patient survival and high-risk tumours, and inversely correlated with MYCN oncogene amplification. Surprisingly, our results indicate that MYCN does not affect the expression of either helicase, but that it does alter transcription termination. This transcription factor directly promotes readthrough transcription and tracRNA production via its binding to the 3' region of genes, and it interacts with DDX17, suggesting that its overexpression in cancer cells could alter the activity of the helicase. Our work reveals a novel function of MYCN in transcription termination and suggests that the combined deregulation of MYCN and DDX17 genes may lead to readthrough-associated transcriptome changes in neuroblastoma. I will discuss the potential consequences of these alterations in cancer cells, but also in the context of a recently described neurodevelopmental syndrome associated to de novo variants of the DDX17 gene (Seaby et al., Brain 2025).

Session 8

Structural basis for human mitochondrial tRNA maturation

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The human mitochondrial (mt) genome is transcribed into two RNAs, containing mRNAs, rRNAs and tRNAs, all dedicated to produce essential proteins of the respiratory chain. Precise excision of tRNAs by the mt-RNase P and Z, releases all RNA species from the two RNA transcripts. The tRNAs then undergo 3′-CCA addition. The low structural stability of mt-pre-tRNAs adds significant complexity to these steps, and defects in their maturation are implicated in various human mt-disorders. In this case, the tRNA methyltransferase complex TRMT10C/SDR5C1 compensates for the pre-tRNA structural alteration to present the pre-tRNA to maturation enzymes. The human mt-RNase P is a multi-enzyme assembly that comprises the endoribonuclease PRORP and a tRNA methyltransferase subcomplex. The requirement for this tRNA methyltransferase subcomplex for mt-RNase P cleavage activity, as well as the mechanisms of pre-tRNA 3′-cleavage and 3′-CCA addition, were still poorly understood. We determined cryo-EM structures that visualise four steps of mt-tRNA maturation : 5′ and 3′ tRNA-end processing, methylation and 3′-CCA addition, and explain the defined sequential order of the tRNA processing steps. The methyltransferase subcomplex recognises the pre-tRNA in a distinct mode that supports tRNA-end processing and 3′-CCA addition, likely resulting from an evolutionary adaptation of mitochondrial tRNA maturation complexes to the structurally-fragile mt-tRNAs. This subcomplex can ensure a tRNA-folding quality-control checkpoint before the sequential docking of the maturation enzymes. Altogether, our study provides detailed molecular insight into RNA-transcript processing and tRNA maturation in human mitochondria. Understanding these maturation steps offers deeper insight into the molecular mechanisms underlying mt-disorders and diseases.

[1] Meynier V. et al. (2024) Structural basis for human mitochondrial tRNA maturation. Nature Communications 15: 4683-4699.
[2] J. Sikarwar J. et al. (2025) Advances in Human Pre-tRNA Maturation : TRMT10C and ELAC2 in Focus. J Mol Biol. 437: 168989-169001.

Session 8

tRNAslational control of eukaryotic gene expression

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My research group investigates various mechanisms of translational control that regulate the production of specific protein sets through chemical modifications of tRNA molecules. In cells, all proteins are synthesized by the ribosome, which relies on transfer RNA (tRNA) to decode the genetic information in mRNA and assemble polypeptide chains accordingly. Our lab focuses on uncovering the molecular mechanisms that drive specific base modifications within the anticodon regions of tRNAs. These modifications significantly impact the efficiency and fidelity of codon-anticodon pairing, thereby influencing translational rates and the dynamics of protein folding. Recent studies have revealed that disruptions in these modification pathways are closely linked to the development of certain neurodegenerative diseases and cancers. To investigate these processes, we primarily employ X-ray crystallography (MX) and cryogenic electron microscopy (cryo-EM) to capture high-resolution snapshots of the macromolecular complexes involved and to visualize reaction intermediates at the atomic level. We complement these structural approaches with a variety of in vitro and in vivo methods to validate and interrogate our findings. In addition to our core focus, we have begun exploring other (t)RNA modification pathways and are now using cryo-EM to directly resolve the structures of folded RNA molecules. We also aim to elucidate how post-transcriptional modifications influence ribosomal decoding and translation elongation by directly imaging translating ribosomes at atomic resolution. Finally, we are developing new structural, biochemical, and biophysical techniques to study structured RNA domains. In summary, our research advances the fundamental understanding of eukaryotic gene expression and its intricate regulatory mechanisms.

Session 8

Which tRNA elements enable tRNA superwobbling?

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tRNAs are essential adapter molecules that decode the genetic code during protein synthesis. Although they share a conserved L-shaped structure, their sequences and chemical modifications vary considerably. RNA modifications influence tRNA stability, folding, structure, and decoding properties. An intriguing group, known as superwobbling tRNAs, can decode all four codons within certain 4-codon boxes. These tRNAs typically contain unmodified uridines at the wobble position, raising the question of how they achieve such broad decoding capability.
To study the molecular basis of superwobbling, we generated various unmodified and modified variants of the superwobbling *Mycoplasma capricolum* $tRNA^{Val}UAC$. Using chemically synthesized RNA fragments combined with splinted ligation, we generated tRNAs carrying different numbers and combinations of modifications and tested their ability to decode all four valine codons. All tested tRNAs - modified and unmodified - efficiently decoded GUU, GUA, and GUG codons. However, none efficiently decoded GUC.
Analysis of the *M. capricolum* genome revealed that GUC codons are strongly underrepresented. Only one gene carries the maximum of four GUC codons, which can still be translated effectively despite the limited decoding capacity of the tRNA. Our findings indicate that $tRNA^{Val}UAC$ is not a fully efficient four-codon superwobbler, but rather a “sufficiently superwobbling” tRNA whose decoding range is tuned to the organism’s codon usage. This aligns with previous work showing that, while some tRNAs can decode all four codons efficiently, others are adapted to more restricted but adequate decoding requirements.

Session 8

tRNA-derived RNAs: Biogenesis, numbers, molecular interactions and functional potential of a cellular 'waste product'

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Stress-induced tRNA fragmentation is an evolutionary conserved process that is catalyzed by endonucleolytic activities targeting mature tRNAs. The resulting tRNA-derived RNAs (tDRs) are detectable in many biological systems, and experimental manipulation indicated that these small RNAs could affect various biological processes.
How highly structured tRNAs give rise to individual and potentially biologically active tDRs remains poorly understood. Specifically, how many specific tDRs are produced per cell; where specific tDRs localize during and after the stress response; do tDRs impact cellular physiology through protein interactions?
Our lab addresses these questions by using and developing biochemical and imaging methodology, with a keen eye on the quantification of molecular numbers as a base for forming and testing specific hypotheses.
In this talk, I will discuss the current state of research into tDR biology, point out numerous misconceptions in the field, and describe our progress in better understanding the biogenesis and functional potential of specific tDRs.

Session 8

tRNA modification in development and disease

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To date, more than 150 RNA modifications have been described, most of which are found in tRNAs. Modifications in the anticodon loop are crucial for protein translation efficiency and fidelity, whereas modifications in the rest of the molecule are thought to be important for folding and structural stability of the tRNA. Remarkably, mutations in many tRNA modification enzymes cause diseases in humans, often characterized by a phenotype restricted to specific tissues, or during development.
Using cellular biology, biochemistry, and NGS-based methods, we investigate the dynamics of tRNA expression and modification during embryo development; furthermore we are shedding light on the impact of the perturbation of tRNA maturation in physiology and disease, with a special focus on pancreatic insulin-secreting beta-cells.

Session 8

Ashwin and FAM98 paralogs define nuclear and cytoplasmic RNA ligase complexes for tRNA biogenesis

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The tRNA ligase complex (tRNA-LC) seals tRNA exon halves in the nucleus during pre-tRNA splicing and joins *XBP1*-mRNA exons in the cytoplasm as part of the unfolded protein response (UPR). This dual function requires the ability to be either nuclear or cytoplasmic. Here, we reveal that Ashwin (ASW), the vertebrate-specific subunit of the tRNA-LC, serves as its nuclear import factor. ASW contains a dual nuclear localisation signal (NLS) which, upon disruption, leads to the retention of the tRNA-LC in the cytoplasm, impairing pre-tRNA splicing with the consequent accumulation of 5' tRNA fragments. We also show that the tRNA-LC exists in three forms, depending on which FAM98 paralog is bound, either FAM98A, FAM98B or FAM98C. ASW interacts exclusively with the FAM98B-containing complex, ensuring its nuclear localization for tRNA biogenesis. Attaching an NLS to RTCB, the catalytic and indispensable tRNA-LC subunit, rescues pre-tRNA splicing in cells depleted of ASW. We hypothesize that vertebrates evolved ASW to localize a sub-population of tRNA-LC to the nucleus, while using FAM98 paralogs to retain a fraction of RTCB in the cytoplasm for *XBP1*-mRNA splicing during UPR.

Session 8

Transfer RNAs: architects of brain development

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The cerebral cortex comprises hundreds of excitatory projection neuron subtypes, all arising from a uniform pool of apical progenitors that sequentially generate neuronal progeny with distinct fates. While transcriptional programs contribute to neuronal diversity, many mRNAs found in mature neurons are already present—but untranslated—in progenitors. This suggests that additional layers of regulation, including translational control, shape neurogenesis.
Translation bridges mRNA presence and protein production, and its efficiency can be dynamically regulated by tRNAs. Notably, altered translational efficiency causes neurodevelopmental defects in mice, and mutations in genes regulating tRNA biology are linked to human neurodevelopmental disorders.
We investigated the role of tRNA abundance and modification in somatosensory cortex development. Using a candidate-based approach, we studied the ADAT2/3 complex, which deaminates tRNA wobble adenosines into inosines. We provide the first in vivo evidence that ADAT activity regulates radial migration of projection neurons. Pathogenic ADAT3 variants impaired complex function and reduced Inosine 34 levels, disrupting tRNA steady state. The severity of migration phenotypes correlated with the degree of tRNA deamination loss.
In parallel, we applied mimtRNA-seq to generate full-length mature tRNA profiles across neurodevelopmental stages. We show that tRNA levels are dynamic and possibly cell type–specific. Our data reveal differential isodecoder contributions to anticodon pools and suggest that regulation of their transcription in maintaining partly ensure proper tRNA availability.
Altogether, we highlight tRNAs as active regulators of cortical development and propose a model where translational control shapes neuronal fate and function.

mRNA binding protein therapeutics for severe muscle injuries, muscle wasting conditions and genetic diseases

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AUF1 is an RNA binding protein that binds repeated AU-rich elements (AREs) located in the 3' untranslated region of approximately 3% of mRNAs, which we have shown in muscle encode the major regulators of muscle maintenance and regeneration (myogenesis), mitochondrial biogenesis and function, and formation of neuromuscular junctions. AUF1 can destabilize certain ARE-mRNAs and stabilize and increase translation of others. We previously showed that sarcopenia, the severe loss of muscle with aging, involves accelerated loss of AUF1 in skeletal muscle with age, and one form of limb girdle muscular dystrophy (type 1G) in humans is caused by mutations in AUF1. Skeletal muscle-specific AUF1 supplementation is therefore an attractive target for the treatment of muscle atrophy in aging, after severe injury, protracted limb immobilization and certain muscular dystrophies.

We developed both AAV8 AUF1 muscle-tropic gene therapy vectors and a highly skeletal muscle-specific lipid nanoparticle (LNP) mRNA formulation (REG-1003) that de-targets liver and spleen for long-term and short-term administration of AUF1, respectively. When systemically administered to very old sarcopenic mice (24 month, ~80 year old human), AUF1 therapy restored muscle morphology, muscle stem cells, muscle mass, functional mitochondria and exercise performance to that of 12 month old mice (~50 year human). Intramuscular or systemic administration of REG-1003 24h after severe muscle injury or limb immobilized disuse atrophy halved the time for muscle regeneration, reversed atrophy and retained normal muscle strength, compared to a 25% loss in untreated animals. Long-term safety data in mice showed no safety concerns, inflammation or neoplastic signals. IND-enabling studies for REG-1003 are currently underway.

Session 10

The Fault in our Vaults: Distinct roles of vault RNA paralogs in liver cancer cells

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Vault RNAs (vtRNAs) are eukaryal non-coding RNAs transcribed by RNA polymerase III and have emerged as regulators of essential cellular pathways, including differentiation, proliferation, apoptosis, and autophagy [1]. Despite these broad functional roles, the molecular mechanisms by which individual vtRNA paralogues contribute to tumorigenesis remain insufficiently understood.
We uncovered distinct functions for vtRNA paralogs, vtRNA1-2 and vtRNA1-1, in orchestrating cell proliferation and tumorigenesis. Loss of either vtRNA1-2 or vtRNA1-1 reduced cell proliferation in HCC cells, and in mouse xenograft models, vtRNA1-1 knockout–derived tumors showed significantly diminished growth and increased chemosensitivity [2]. We identified a previously uncharacterized role for vtRNA1-2 in regulating angiogenesis. In Huh7 liver cancer cells, vtRNA1-2 modulates the secretion of angiogenic factors, resulting in changes of angiogenic activity of endothelial cells [3]. These findings indicate vtRNA1-2 as a regulatory node influencing the tumor microenvironment.
In contrast to this angiogenic role of vtRNA1-2, vtRNA1-1 displays a mechanistically distinct contribution to cancer cell hallmarks supported by transcriptomic data. Phosphoarray profiling of vtRNA1-1 knockout cells uncovered broad activation of kinase pathways associated with stress and pro-survival signaling, including alterations in mTOR, MAPK, and NF-κB pathways. vtRNA1-1 knockout cells additionally exhibit decreased global translation, elevated reactive oxygen species levels, and enhanced sensitivity to ferroptosis, indicating disrupted redox homeostasis. To uncover the mechanistic basis of these effects, we identified vtRNA1-1 protein interactors using RAP-MS. The top candidate interactors are linked to stress adaptation and metabolic regulation, revealing how this ncRNA engages key regulatory networks that shape tumorigenic signaling.

[1] S. Gallo, E. Kong, I. Ferro, and N. Polacek, “Small but Powerful: The Human Vault RNAs as Multifaceted Modulators of Pro-Survival Characteristics and Tumorigenesis,” Jun. 01, 2022, MDPI. doi: 10.3390/cancers14112787.
[2] I. Ferro et al., “The human vault RNA enhances tumorigenesis and chemoresistance through the lysosome in hepatocellular carcinoma,” Autophagy, vol. 18, no. 1, pp. 191–203, 2022, doi: 10.1080/15548627.2021.1922983.
[3] S. Gallo et al., “Regulation of angiogenesis and cancer cell proliferation by human vault RNA1-2,” NAR Cancer, vol. 7, no. 3, Sep. 2025, doi: 10.1093/narcan/zcaf028.

Session 10

Decoding lncRNA-mediated Regulation of Macrophage Function in Inflammation and Repair

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The heart's intricate cellular landscape includes cardiac resident macrophages (CRM) as important players in cardiac health and disease. On a molecular level, long non-coding RNAs (lncRNAs) emerge as intriguing regulators, given their capabilities in influencing cell identity, proliferation, and inflammation. Despite their known importance in cardiac myocytes and other cardiac cell types, lncRNAs in CRMs have not been characterized in detail yet.
We investigated the role of long noncoding RNAs (lncRNAs) in regulating CRMs in health and disease. We selected thirty conserved and disease-associated lncRNAs to uncover their functions in macrophage activation, inflammation, and tissue repair. Using high-throughput CRISPR-based screening (CRISPR-droplet sequencing, CROP-Seq) and transcriptomic profiling of the single cell sequencing data, we identified several lncRNAs that significantly caused a transcriptomic phenotype. Among them, NIP16 emerged as a key regulator of pattern recognition receptor pathways, including the CLEC4 family. These pathways mediate immune responses to tissue damage and infection. Functional studies revealed that NIP16 modulates inflammatory signaling and may affect cardiac repair processes following injury. Ongoing experiments in mouse knockout models and human iPSC-derived macrophages aim to define NIP16's contribution to sterile inflammation and post-injury regeneration. Together, this work seeks to uncover fundamental mechanisms by which lncRNAs shape macrophage behavior and to explore their potential as therapeutic targets in inflammatory and cardiovascular diseases.

Session 10

Paternal loss of pi6 piRNAs disrupts the early embryogenesis in mice

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At fertilization, gametes transmit both genetic and epigenetic information to embryos for successful embryogenesis. Unlike the maternal epigenetic contribution, the role of paternal epigenetic factors in this process remains poorly understood. Sperm small RNAs, including tRNA fragments, miRNAs and PIWI-interacting RNAs (piRNAs), have been proposed to mediate intergenerational paternal influences. However, the mechanism remained unknown, partly because of a lack of a suitable animal model. In this study, we used the pi6em1/em1 mouse model to better understand the mechanism of paternally inherited small RNAs in embryos. The pi6em1/em1 mouse, which lacked a sub-population of piRNAs from the pi6 locus, was male subfertile and demonstrated an unexpected paternal phenotype, suggesting a post-fertilization role for mouse piRNAs [1]. Most embryos fertilized with pi6em1/em1 sperm were arrested before the blastocyst stage, especially at the one- or two-cell stages, coinciding with the typical timing of zygotic genome activation (ZGA). To determine the underlying cause of the paternal pi6em1 embryo phenotype, we analyzed the transcriptome of single zygotes and two-cell embryos fertilized by pi6em1/em1 or wild-type sperm by RNA-seq. Unexpectedly, maternal mRNA clearance and zygotic mRNA accumulation were accelerated, and mRNAs encoding protein components of the mitochondrial oxidative phosphorylation pathway were disproportionally affected in paternal pi6em1 embryos. Together, our data indicated that an accelerated—not delayed—gene expression program that led to ill-timed ZGA and metabolism was detrimental to paternal pi6em1 embryos.

[1] Wu, P.-H. et al. The evolutionarily conserved piRNA-producing locus pi6 is required for male mouse fertility. Nat Genet 52, 728–739 (2020).

Session 10

Effector-mediated functional switching of PETISCO between piRNA processing and histone mRNA storage

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PETISCO is a multi-protein complex initially identified as an essential factor in *Caenorhabditis elegans* piRNA biogenesis. However, genetic evidence has long indicated a second, piRNA-independent role for PETISCO critical for early embryogenesis. Recent data from the Ketting lab show that PETISCO also helps to store replication-dependent histone mRNAs during oogenesis.
Two mutually exclusive effector proteins determine the activity of PETISCO: PID-1 is essential for PETISCO-dependent piRNA processing, whereas TOST-1 redirects PETISCO to histone mRNA storage. However, the mechanism by which effector-mediated specificity is established has remained unresolved. We show that TOST-1 licenses PETISCO to selectively bind histone mRNAs by recognizing the conserved stem–loop present at the 3‘-end of replication-dependent histone mRNAs.
Moreover, we identify an additional PETISCO-binding region in TOST-1, which blocks PETISCO from associating with the piRNA biogenesis machinery, thus preventing unwanted processing of histone mRNAs. Structural and biochemical analyses reveal that a short helical motif in TOST-1 binds the SN-like part of the TOFU-6 extended Tudor (eTUDOR) domain, a binding surface also used by piRNA biogenesis factors. High-throughput AlphaFold-based interaction screenings identify additional regulators targeting this eTUDOR region, indicating that it constitutes a competitive interaction hotspot that defines PETISCO localization and pathway specificity.
Together, these findings establish the first mechanistic model of effector-mediated PETISCO specification and reveal how TOST-1 not only enables histone mRNA storage but simultaneously shields maternal histone mRNAs from piRNA-directed RNA processing.

Establishment of a human iPSC model of inflammatory diseases for drug development

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Inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease, pose significant challenges to both patients and healthcare systems worldwide. Understanding the underlying mechanisms driving inflammation is essential for the development of effective treatments. In this study, we present the establishment and characterization of a human induced pluripotent stem cell (hiPSC)-based model to investigate the pathogenesis of inflammatory diseases, where primary material access is limited.
iPSCs hold great promise for disease modelling due to their ability to self-renew, to differentiate into various cell types and recapitulate in vitro phenotypes of diseases. We employ CRISPR-Cas9 on a human iPSC line to create knock-outs of genes linked to inflammatory diseases. These lines can then be further modulated with lipid nanoparticles containing wild-type or mutant mRNA of the genes of interest to study the protein function in a personalized manner.
We optimized a directed differentiation protocol starting from hiPSCs, which we can apply in both wild-type and gene-edited cells generating healthy and disease-related macrophages, respectively. We chose to study macrophages considering their pivotal role in orchestrating the inflammatory response and, therefore, the most likely candidate cells to provide valuable insights into disease pathophysiology.
We characterised the generated cells by surface marker expression and transcriptomic analysis to elucidate gene expression profiles and functional networks associated with inflammation in our hiPSC-derived cell models. Our model enables the study of diverse aspects of macrophage function in both basal and inflammatory context, including phagocytosis, cell migration and cytokine secretion after stimulation with different inflammatory and anti-inflammatory cytokines.

Edited Filamin A in myeloid cells reduces intestinal inflammation and protects from colitis

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The patho-mechanistic origins of inflammatory bowel diseases are still poorly understood. The actin crosslinking protein Filamin A (FLNA) impacts cellular responses through interactions with numerous, cytosolic proteins, thereby crosslinking actin and serving as a hub for cellular signaling. One amino acid of FLNA within a highly interactive region is changed from the genome-encoded FLNAQ to an isoform, created upon A-to-I RNA editing, resulting in FLNAR. Using mouse models with unedited FLNAQ or fully-edited FLNAR, we identified this editing as a key determinant of colitis severity. Editing was highest in healthy colons and reduced during murine and human colitis. Mice that exclusively express FLNAR, were resistant to DSS-induced colitis, whereas unedited FLNAQ animals developed severe inflammation. While FLNA editing influenced intestinal structural cells and microbiome composition, we found that FLNAR exerts protection specifically via hematopoietic cells – mainly myeloid cells. Key effector functions of FLNAR neutrophils were changed compared to unedited cells. Upon characterization of FLNA editing levels throughout the mouse colon, we found low FLNA editing in all major immune cell subsets. Thus, the induction of high FLNA editing levels in myeloid cells correlated with protection from severe colitis, which could be therapeutically exploited by targeted FLNA editing in the future.

https://doi.org/10.1084/jem.20240109

Extrahepatic delivery of siRNA by nonviral protein cages

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Nucleic acid-based therapeutics have the potential to transform medicine through precise control of gene expression, but their impact beyond the liver is constrained by a central hurdle: selective and efficient uptake into defined tissues or cell types. Nature overcomes these barriers with protein-based delivery vehicles such as viruses.
We introduce a nonviral delivery platform designed to capture the functional strengths of viral systems through robust packaging, protection, and intracellular delivery of nucleic acids: an engineered protein cage optimized for nucleic acid encapsulation and delivery [1].
We apply a rational design strategy to overcome the challenges of cargo loading and endosomal entrapment. By introducing arginine residues on the cage lumen, we generate a positively supercharged interior that binds and encapsulates negatively charged oligonucleotides with high affinity [2]. siRNA-loaded cages are efficiently internalized by mammalian cells, but they initially remained sequestered in endosomes. Modification of the cage surface with saponins enables endosomal escape into the cytosol, facilitating successful cargo release to induce RNA interference.
Using this engineered system, we demonstrate efficient in vivo delivery of siRNA to extrahepatic tissues that have historically been difficult to target, including the adrenal gland, where we achieve potent and durable gene silencing. As a first therapeutic application, we modulate circulating corticosterone levels in vivo for the treatment of hypercortisolism.
In summary, we present a fully engineered protein cage capable of tissue-specific, in vivo siRNA delivery. Ongoing work aims to elucidate and refine the determinants of tissue targeting to broaden delivery to additional cell types.

[1] Edwardson TGW, Levasseur MD, Hilvert D. The OP Protein Cage: A Versatile Molecular Delivery Platform. Chimia (Aarau). 2021 Apr 28;75(4):323-328. doi: 10.2533/chimia.2021.323. PMID: 33902803.
[2] Edwardson TGW, Mori T, Hilvert D. Rational Engineering of a Designed Protein Cage for siRNA Delivery. J Am Chem Soc. 2018 Aug 22;140(33):10439-10442. doi: 10.1021/jacs.8b06442. Epub 2018 Aug 9. PMID: 30091604.

Session 11

ASO technology for phage functional genomics and therapy

VOGEL, Joerg

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Antisense oligonucleotides (ASOs) are the most successful molecule class in RNA-based medicine, but they are also attracting ever more attention for applications outside, especially in microbes [1]. The natural enemies of bacteria are phages, these constitute the most abundant biological entity on earth and exhibit vast genetic and phenotypic diversity. Exploitation of this largely unexplored molecular space requires identification and functional characterisation of genes that act at the phage-host interface. This has been restricted to a few model phage-host systems that are amenable to genetic manipulation. To overcome this limitation, we have developed a non-genetic mRNA targeting approach using exogenous delivery of ASO to silence genes of both DNA and RNA phages [2]. We applied these ASOs for a systematic knockdown screen of core and accessory genes of the nucleus-forming jumbo phage ΦKZ, coupled to RNA-sequencing and microscopy analyses. This revealed previously unrecognised proteins that are essential for phage propagation and that, upon silencing, elicit distinct phenotypes at the level of the phage and host response. I will discuss how our non-genetic ASO-based gene silencing promises to be a versatile tool for molecular discovery in phage biology, will help elucidate defence and anti-defence mechanisms in non-model phage-host pairs, and offers potential for optimising biotechnological procedures and phage therapy.

[1] Vogel J, Faber F, Barquist L, Sparmann A, Popella L, Ghosh C (2025) Meeting report ASOBIOTICS 2024: an interdisciplinary symposium on antisense-based programmable RNA antibiotics. RNA 31(4):465-474
[2] Gerovac M, Buhlmann L, Zhu Y, Đurica-Mitić S, Rech V, Carien S, Gräfenhan T, Popella L, Vogel J (2025) Programmable antisense oligomers for phage functional genomics. Nature 646(8087):1195-1203

Session 11

SMG6-dependent nonsense-mediated decay maintains dsRNA homeostasis and suppresses immunogenic transcripts in a hepatocellular carcinoma model

ARPA, Enes Salih (1), RICCI, Virginie (1), SHAPIRO, Ilja E. (2), JOKIC, Marija (3), MOHAMMADZADEH, Yahya (4), BERTRAND, Lisa (1), GONZALEZ-MENENDEZ, Irene (5), QUINTANILLA-MARTINEZ, Leticia (5), BENECHET, Alexandre P. (6), REHWINKEL, Jan (7), PETROVA, Tatiana V. (4), LUTHER, Sanjiv A. (3), BASSANI-STERNBERG, Michal (2), GATFIELD, David (1)

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The nonsense-mediated mRNA decay (NMD) pathway degrades transcripts bearing premature termination codons (PTCs), typically arising from mutations and RNA processing errors, to prevent the accumulation of aberrant proteins. Its role in cancer is complex, with both tumor-promoting and tumor-suppressive functions described. Here, we selectively inhibit NMD in the liver by conditionally inactivating its endonuclease SMG6 in a genetic mouse model of hepatocellular carcinoma. SMG6 inactivation completely abrogates tumor formation and elicits both innate and adaptive immune responses. Mechanistically, SMG6 deficiency activates the type I interferon response via the double-stranded RNA (dsRNA) sensor MDA5, uncovering a physiological role for NMD in regulating cytoplasmic dsRNA levels. In parallel, stabilization and translation of NMD-target transcripts generate non-canonical immunopeptides presented by MHC-I, promoting potent CD8+ T-cell responses. These findings establish SMG6-dependent NMD as a core orchestrator of immune tolerance in cancer and reveal that its selective inhibition in cancer cells unleashes immunogenic transcript expression with therapeutic potential.

Session 11

Mechanistic insights of RNA toxicity and disrupted RNA processing from modeling Myotonic Dystrophy in mice

PETERSON, Janel AM (1,2), FRIAS, Jesus A (1), SONI, Krishnakant G (5), DAY, John W (6), PREIDIS, Geoffrey A (5,7), COOPER, Thomas A (1,3,4)

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Myotonic dystrophy type I (DM1) is the most common adult-onset muscular dystrophy, clinically affecting one in 8000 people with a mutation frequency of 1 in 2100 in the United States. DM1 causes variable multisystemic symptoms severely affecting the brain, heart, skeletal muscle, and gastrointestinal (GI) tract. The DM1 mutation is a CTG repeat expansion in the 3′ untranslated region of the *DMPK* gene and pathogenesis is caused by the RNA from the expanded allele containing expanded CUG repeats (CUG_{exp} RNA). CUG_{exp} RNA sequesters the muscleblind-like (MBNL) family of RNA binding proteins, which leads to mis-regulated alternative splicing and expression of protein isoforms that are incompatible with adult tissue function. We have established mouse models to investigate the mechanisms of DM1 pathogenesis in heart, skeletal muscle, brain and the GI track. While >80% of people living with DM1 experience disturbances in GI function due to pan-GI dysmotility, the mechanism of dysfunction has not been investigated. Using a smooth muscle specific conditional knock out of *Mbnl1* and *Mbnl2*, we established a mouse model that reproduces physiological and molecular features consistent with GI dysmotility in DM1. Counterintuitive to clinical conventional wisdom that DM1 GI dysfunction results from poor smooth muscle contractility, our results show increased baseline smooth muscle tone that compromise GI function. The results suggest that medications that reduce GI smooth muscle activity may improve GI symptoms in DM1.

LIST OF POSTERS – OVERVIEW

Poster Session I: Tuesday, January 27, odd-numbered posters
Poster Session II: Wednesday, January 28, even-numbered posters
For more information on the poster sessions, please consult p. 8 of this booklet.

Nr	Title of the poster	Presenter	Group
1	LUTHOR HD – unparalleled sensitivity in subcellular sequencing *(sponsored poster)*	PASSARDI, Filippo	Lexogen
2	Ancient origins for the METTL3 m^6A RNA methylation machinery	AL-LAHIB, Nancy	Pillai
3	Puf3 contributes to changes in mRNA solubility, translation elongation dynamics at rare arginine codons and loss of protein homeostasis in cells lacking Not4	AUDEBERT, Léna	Collart
4	Reconstitution of human translation elongation complexes	BALA KRISHNAN, Rakhshana	Klaholz
5	Morpholino-modified ribozymes: Towards cell-stable catalytic RNAs	BANERJEE, Arpan	Höbartner
6	From protein-repeat RNA interactions to novel treatments of C9-ALS/FTD and Myotonic Dystrophy 1	BARBASH, Daria	Allain
7	Dimerization of ADAR1 modulates site-specificity of RNA editing	BARRAUD, Pierre	Tisné
8	Development of a live single-molecule imaging method to visualize IRE1 oligomerization during mRNA splicing and decay in the UPR	BAYRAK, Göksu	Voigt
9	LIN28 inhibition by small molecule reverses miRNA expression and disease hallmarks in myotonic dystrophy	BEHERA, Alok	Hall
10	Ψ-Spinach: a light-up aptamer functional with total replacement of uridine by N1-methylpseudouridine	BERTRAND, Patrick	Ryckelynck
11	The role of NUDIX enzymes in cellular metabolism and non-canonical mRNA capping	BIRBAUMER, Tosca	Leidel
12	Harnessing epitranscriptomics to detect and manage pancreatic cancer	BRUNEL, Kelly	David
13	Evolution of dosage compensation mechanisms in insects	BRUNNER, Jana	Keller-Valsecchi
14	Immunopeptidome and proteome responses to SMG-1–mediated NMD inhibition in NSCLC show a potential transition toward a hot tumor phenotype	BURGER, Alexandra Emanuela	Heller
15	N^6-methyladenosine (m^6A)-mediated translation control of DGKk by FMRP governs diacylglycerol-dependent neuronal translation and activity	CAKIL, Oktay	Moine
16	The RNA methyl-transferase enzyme FTSJ1: conserved role in neuron morphology & learning performance	CARRÉ, Clément	Carré
17	When neuronal box C/D snoRNAs meet genomic Imprinting: Insights into Prader-Willi Syndrome	CAVAILLE, Jérôme	Cavaille
18	A study of RNA as a molecular memory system: new perspectives from long-lived RNAs	CHAMOT, Anna	Chamot
19	Gene specific splicing correction with small molecules: fundamental insights into gene selectivity and rational design	CHIARAZZO, Giulia	Campagne
20	Lucid platform: A Lab Unified Computational Integration and Discovery platform for data management and enhanced collaboration	CIAUDO BEYER, Constance	Lucid Analytics
21	Studying protein-RNA interactions? The NMR platform can help you out!	CLERY, Antoine	Allain / Platform
22	Investigating the kinetic difference in translation termination at stop codons of NMD-sensitive versus NMD-insensitive transcripts	DAS, Anupam	Mühlemann
23	Metabolic regulation via m^6A_m cap modification of mammalian mRNAs	DELFINO, Elena	Pillai
24	Decoding recruitment: linking splicing arrest to mRNP accumulation in nuclear speckles	DÖRNER, Kerstin	Hondele
25	Investigating the interactions of MDA5 and LGP2 with self-RNA	EHTREIBER, Wanja	Bernecky
26	RNA orchestrates hnRNP condensation and directs 40S hnRNP ribonucleosome assembly	ESCURA PÉREZ, Maria	Allain
27	Bacterial 70S Scanning - An overlooked yet crucial regulatory mechanism for translation initiation?	FASNACHT, Michel	Moll
28	Molecular Architecture of an Endogenous Mouse PIWI protein	FERNANDEZ-RODRIGUEZ, Carmen	Pillai
29	Protein interaction network of human m^5C RNA methyltransferases	FICKL, Magdalena	Lusser
30	Image-Based Discovery of a Recurrent Tumor Archetype Characterized by Aberrant RNA Splicing and Associated with Poor Survival in Breast Cancer	FOURNIER, Lisa	Luisier
31	Correcting a pathogenic G>A point mutation in SBF1 gene by Site-directed RNA editing	FRUGIS, Petronilla	Jantsch

Nr	Title of the poster	Presenter	Group
32	Polysomes and mRNA control the biophysical properties of the eukaryotic cytoplasm	GADE, Vamshidhar	Weis
33	Investigating ASOs as a non-genetic tool for Lactobacilli	GEBLER, Victoria	Vogel
34	Tuning lipid nanoparticle coronas through statistical copolymers for cell-specific gene expression	GEROLIMETTO, Giorgia	Meinel
35	Sustained miR-802 expression redirects KRAS-driven neoplastic fate toward IPMN-like lesions	GIURIATTI, Pietro	Stoffel
36	The role of RNA modifications in the superwobbling $tRNA^{Val}$ of *M. capricolum*	GONNELLA, Isabell	Erlacher
37	Understanding the early stages of Coronavirus infection	GONZALEZ BURGOS, Martin	Thiel
38	Characterization of a M. tuberculosis specific GpsI (RNase) inhibitor	GRIESSER, Tizian	Sander
39	Investigate the role of RNA binding proteins in kidney development and repair using single-cell CRISPR screening and kidney organoids.	GRIFFITHS, Jacob	Welte
40	Determining the impact of non-coding RNA fragments on innate immune signaling	GRUBER, Livia	Schaefer
41	Towards the creation of a comprehensive map of pseudouridine in rRNA & H/ACA box snoRNAs in *Drosophila melanogaster*	GUILLEN ANGEL, Maria	Roignant
42	Exploring biomolecular condensates in bacteria: CsdA forms a nucleoid-associated ribosome biogenesis hub in *E. coli*	GUT, Michelle	Hondele
43	Experimental identification of preQ1-binding RNAs in the pathogenic bacterium *L. monocytogenes*	HANISCH, Malou	Lusser
44	A single rRNA-modifying enzyme modulates ribosome fidelity, stability, and tobramycin tolerance in *Vibrio cholerae*	HARDY, Léo	Baharoglu
45	Biochemical and Structural Studies of eukaryotic pre-mRNA 3'end processing	HAUCK, Theresa C.	Jinek
46	Transcriptome-wide detection of differential RNA editing using LoDEI	HEISE, Tilman	Corbacioglu
47	Deciphering the role of SUMOylation during the programmed genome rearrangement in *Paramecium tetraurelia*	HOGG, Robin	Nowacki
48	Impact of A-to-I editing on m^6A deposition	HONARMAND TAMIZKAR, Kasra	Jantsch
49	ZCCHC9 is a human SSU processome factor required for ENP1 loading and nucleolar 18S rRNA maturation	HORVÁTH, Bianka	Kutay
50	Tumour mutations remodel oncogenic non-coding RNA:protein networks	JOHNSON, Rory	Johnson
51	How do TERRA R-loops promote homology-directed repair at telomeres?	JUNG, Misun	Lingner
52	DarT2 ortholog mining yields flexible Append Editors	KAMM, Charlotte	Beisel
53	Imaging HIV-1 transcription in live latent T cells reveals rare viral transcriptional bursts	KARAKI, Hussein	Bertrand
54	SARS-CoV-2 Nucleocapsid relies on Phase Separation to selectively form viral RNPs inside Condensates	KATHE, Nina	Allain
55	High-resolution mapping of rRNA modifications in *Bacillus subtilis*	KAZAN, Ramy	Tisné
56	Computational approaches for studying readthrough transcripts biogenesis and functions in neuroblastoma cells	KHOURAB, Lou-Sahra	Bourgeois
57	Polyamines and codon-specific translation regulation in cancer	KIENAST, Sandra D.	Morscher
58	SRSF1 condensates enhance splicing by two mechanisms: RNA protection and surface exposure	KOCIOLEK, Noémie	Allain
59	Extended Shine-Dalgarno motifs govern translation initiation in *Staphylococcus aureus*	KOHL, Maximilian P.	Marzi
60	On the chemical mechanism of the twister-sister ribozyme	KOTTERSTEGER, Jonas	Micura
61	Characterization of Translating and RTC-associated Nsp3 in Early CoV Infection	KRATZEL, Annika	Thiel
62	Codon usage and tRNA modifications in the regulation of quiescence	KÜNNE, Annika	Weis
63	Liquid chromatography – mass spectrometry analysis of RNA modifications	KUSNIERCZYK, Anna	Leidel / Platform
64	Programmable Gene Silencing in *Enterococcus faecalis*: From Genetic Toolkit to Next-Generation ASObiotics	LAU, Vincent	Vogel
65	Investigating dynamics of tRNA abundance and modification during embryo development	LAURIN, Josef	Vilardo
66	Unraveling the mechanisms by which plant and synthetic small non-coding RNAs direct gene silencing in bacteria	LEBEAU, Liam	Navarro
67	Understanding the physiological and pathological roles of the tRNA deaminase complex ADAT2/ADAT3 during cortical development	LECAT, Romain	Godin

Nr	Title of the poster	Presenter	Group
68	Regulatory mechanisms of mRNA sequestration and fate in P-bodies	LESSLAUER, Aurèle Sylvestre	Spang
69	Deciphering the functional interactions between RNA-binding proteins and the miRISC complex	LISON, Mateo	Jouravleva
70	RNA-PROTACs in ALS: Toward Selective Degradation of TDP-43	LOMBARDI, Ivan	Polymenidou
71	RNA binding and condensate formation by translation initiation factor eIF4B	MACKERETH, Cameron	Mackereth
72	Mass spectrometry analyses of pre-ribosomal 40S intermediates reveal a recruitment platform for processing enzymes	MARCHAND, Désirée	Jonas
73	SINV Infection and Oxidative Stress Trigger Dicer Redistribution to Cytoplasmic Foci in *Myotis myotis* cells	MARIE, Hugo	Pfeffer
74	Optimizing site-directed RNA editing of Filamin A as a therapeutic approach for cardiovascular disease, colitis, and vascularized tumors	MARQUES, Ângela	Jantsch
75	The m^5C RNA methyltransferase Nsun6 regulates neurodevelopment in mammalian and Drosophila models	MARTINEZ, Carlos	Roignant
76	The role of Gigyf1 in pancreatic β-cell signaling	MAYRHOFER, Johanna	Stoffel
77	Targeting the transcription system of poxviruses for antiviral drug discovery	MEEL, Pranjal	Fischer
78	A Self-supervised Morphological Atlas from Fluorescence Microscopy reveals Astrocyte Plasticity in Amyotrophic Lateral Sclerosis	MESSORI, Elisa	Luisier
79	Transcriptomic characterization of a novel iPSC reference cell line for single molecule mRNA imaging	MESTRE FOS, Santi	Chao
80	Modeling human Argonaute-1 mutations associated with intellectual disability in *Drosophila melanogaster*	MEZIANE, Nassim	Carré
81	Improved methods for translation site imaging and visualization of single RNA-binding proteins in live cells	MISIASZEK, Agata	Chao
82	Linking translation dynamics and protein homeostasis in human degenerative disease	MÜLLER, Jan	Leidel
83	RNA decay and KSHV infection: Deciphering the viral-host arms race to control RNA stability	MULLER, Mandy	Muller
84	Maternal age modulates early embryonic translation to influence developmental competence in zebrafish	NABIH, Amena	Pauli
85	Enhancer lncRNA LOC730338 modulates BCR signaling and immune evasion in lymphoma by regulating RNA homeostasis.	NAPOLI, Sara	Bertoni
86	UPF1 shuttles between nucleus and cytoplasm independently of its RNA-binding and ATPase activities	NASIF, Sofia	Mühlemann
87	The m^6A modification and translational adaptation	NENADOVIC, Milena	Moll
88	Antisense oligomers mitigate the genotoxic activity of colibactin-producing *Escherichia coli*	NENTWICH, Sarah	Vogel
89	High-pressure NMR reveals stress-induced changes in multicomponent biomolecular condensates	NOVAKOVIC, Mihajlo	Allain
90	A role for mRNA translation control in oxytocinergic signaling and social behavior adaptations	ODERMATT, Julia	Scheiffele
91	Exploring roles of TnpB beyond transposition control in *Helicobacter pylori*	PANJA, Shounok	Sharma
92	Synthetic mRNA Platform	PENNA, Rocco Roberto	Pascolo / Platform
93	The role of Nonsense-mediated mRNA decay in early mammalian development	PETER, Franziska Elisabeth	Mühlemann
94	Pooled CRISPR Screens identify a lncRNA involved in the innate inflammatory Immune Response	PETZOLD, Niklas	Dueck
95	High throughput protein crystallization platform	PFLEIDERER, Moritz	Jinek
96	Towards covalent fluorescent light-up aptamers (coFLAP) for RNA imaging	PICHLER, Alexander K.	Micura
97	The eIF3-Gpbp1 axis: a paradigm for eIF3-mediated repression?	POETZ, Fabian	Bühler
98	Structural characterisation of RNA-Edited FLNA/FLNB Ig22 Domains	RAJENDRA, Vinod	Jantsch
99	Synthetic strategies for diverse chemical modifications of antisense oligonucleotides	RAPPERT, Dominik	Höbartner
100	The role of cellular condensate maturation in gene expression	RÄSCH, Felix	Weis
101	Mapping sites of RNA oxidation in aged tissues	RIEN, Jakob	Zavolan
102	Impact of tRNA m^1G9 methylation in insulin-secreting β-cells	ROCCHEGIANI, Anna	Vilardo

Nr	Title of the poster	Presenter	Group
103	RNA levels determine stress-specific P-body properties	ROMMEL, Madeleine; MOOKHERJEE, Debdatto	Spang
104	The Structured RNA-binding Domains and Condensation Capacity of FUS Shape its RNA-binding Landscape and Function	RUEPP, Marc-David	Ruepp
105	*Mycobacterium abscessus* Eis2 is an aminoglycoside resistance determinant and pro-drug activator of ribosomal antibiotics	SANDER, Peter	Sander
106	Characterization of the Not condensates and their role in regulation of translation elongation dynamics	SARKAR, Lona	Collart
107	Isolation of endothelial extracellular vesicles from plasma as a basis for RNA biomarker discovery in sickle cell disease	SAUER, Marina	Sommer
108	Condensation of repeat-expansion RNA requires dynamic RNA remodeling that is chaperoned by muscleblind-like splicing regulators	SCHMOLL, Johannes	Allain
109	A human cell-free translation screen identifies the NT-2 mycotoxin as a ribosomal peptidyl transferase inhibitor	SCHWALLER, Nino	Karousis
110	Single-molecule dissection of pathogenic AGO2 variants implicated in Lessel–Kreienkamp syndrome	SEIMEL, Mario	Grohmann
111	Function of m^{6}A mRNA modification during meiosis in yeast	SÈNE, Lina	Seraphin
112	NCCR RNA and disease bioinformatics platform	SHARMA, Puneet	Kutay / Platform
113	Understanding the assembly and function of the 40S hnRNP ribonucleosome	SINGH, Jitendra	Mühlemann
114	Determining the RNA Interactome of Glycolytic Enzymes	SMIRNOVA, Uliana	Schaefer
115	Integrating modified nucleotides into RNA secondary structure predictions	SPICHER, Thomas	Hofacker
116	Rational design of RNA-guided molecular tools for the precise integration of therapeutic DNAs	STEFANOV, Bozhidar-Adrian	Nowacki
117	Investigating the Medical Potential of Alternative splicing in Cancer Therapies	STEUER, Jakob	Kahraman
118	Engineering AI-guided LNPs for cell-specific mRNA delivery	SUGDEN, Maya	Grünewald
119	Beyond Bulk: How scRNAseq, 3' UTR length and alternative splicing contextualises and advances bulk transcriptomic analysis	TANG, Simon	Luisier / Fellay
120	RBMX functional retrocopy safeguards brain development	TILLIOLE, Pierre	Godin
121	Localized translation at the nuclear pore complex	TOTH, Robert	Collart
122	Characterization of new variants in untranslated regions in Diamond-Blackfan anemia syndrome	TROALEN, Paul	Gleizes
123	Translational control of cell response to histidine depletion in T-cell acute lymphoblastic leukemia	ULRICH, Simona	Morscher
124	Decoding tRNA structural information from chemical probing data	VELANDIA-HUERTO, Cristian Arley	Hofacker
125	Geometric deep learning for context-aware protein interactomes	VINCENT-CUAZ, Cédric; THOMAS, Alois	Luisier
126	RNA synthesis platform: Beyond specialized oligonucleotide service	VINCENT, Mathilde	Hall / Platform
127	Early response to warm temperature in arabidopsis	VUARAMBON, Dominique	Reis
128	Beyond nuclear export: UPF1's N-terminus regulates UPF2 and UPF3B levels	WAHL, Evelin	Mühlemann
129	Interplay Between rRNA Expansion Segments and rancRNAs in Translation Regulation in *Saccharomyces cerevisiae*	WAI, Hsu Lei	Polacek
130	Poly(2-oxazoline) lipids as PEG alternatives: modulating LNP surface structure	WANG, Xiaoxuan	Meinel
131	Design and Synthesis of TDP-43 targeting RNA-PROTACs	WELLER, Céline	Hall
132	High-content drug screens identify novel regulators of mRNA localization	WILLIAMS, Tobias	Chao
133	Codon-specific regulation of translation by polyamines independent of eIF5A hypusination	YUAN, Yuan	Morscher
134	The function of non-coding RNAs in the neurophysiological profile of Williams-Beuren Syndrome	ZAVOGIANNI, Niki	Schratt
135	Intersections of NMD and MDA5 in cellular dsRNA surveillance	ZHANG, Emma	Gatfield
136	TRIM71-Congenital Hydrocephalus: The Impact of RNA-binding Protein Dysfunction in Brain Development	GOULOIS, Alison	Grosshans

POSTER ABSTRACTS

Poster 1 (Sponsored Poster)

LUTHOR HD – unparalleled sensitivity in subcellular sequencing

PASSARDI, Filippo

Lexogen (Austria)

A key quality metric in single-cell RNA-seq is the ability to capture all RNA molecules present in each sample fraction. Here we introduce the novel LUTHOR HD 3' mRNA-Seq protocol based on THOR amplification that holds the power to capture mRNA transcripts close to the estimated maxima. THOR technology can be used to generate individual and pooled 3' mRNA-seq, or full-length individual libraries from ultra-low input RNA, cytosolic cell fractions or lysed single cells. The technology is template-switch- and ligation-free, and employs a unique THOR reaction. The THOR reaction is initiated at oligo(dT) primed poly(A) tails introducing a T7 promoter sequence to all 3' ends of transcripts. The resulting structure allows swift amplification of antisense RNA directly from mRNA templates. With the help of LUTHOR HD we accessed the sequencing depth detection limits for single cell experiments, and input amounts as low as 1 pg total RNA equivalents. Further, we investigated R^2 calculations which define correlations of detected but also non-detected genes in replicates taking the statistical constraints through read depth limitations into consideration. Such metrics are needed to distinguish experimental noise from expression data in cytosol sampling.

Poster 2

Ancient origins for the METTL3 m^{6}A RNA methylation machinery

AL-LAHIB, Nancy (1), FERNANDEZ-RODRIGUEZ, Carmen (1), UCHIKAWA, Emiko (2), HOMOLKA, David (1), PILLAI, Ramesh (1)

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More than one hundred different chemical modifications decorate cellular RNAs, with N^6-methyladenosine (m^{6}A) being the most abundant internal modification in eukaryotic mRNAs. m^{6}A is deposited on the consensus DRACH motif (R = A or G; H = A, U, or C) and is enriched near stop codons and 3′ untranslated regions, where it regulates diverse physiological processes across eukaryotes. Through YTH-domain reader proteins, m^{6}A influences RNA stability, translation, splicing, and nuclear export. Installation of m^{6}A is mainly mediated by the METTL3–METTL14 heterodimer (MAC; m^{6}A -METTL complex), in which METTL3 provides catalytic activity and METTL14 contributes to substrate recognition. Although MAC methylates DRACH motifs in vitro, its in vivo specificity depends on additional regulatory factors within the larger m^{6}A writer complex, composed of MAC and the essential regulatory subcomplex MACOM (m^{6}A -METTL-associated complex).
Here, we determined a 2.9 Å cryo-electron microscopy structure of sponge MACOM, revealing its molecular organization and demonstrating strong structural conservation from sponge to human. Cross-linking mass spectrometry further shows that MACOM undergoes substantial remodelling upon binding MAC and that MACOM forms a dimer. Together, these data provide insight into how MACOM is assembled and how it may interact with MAC within the m^{6}A writer complex, establishing a structural foundation for future mechanistic studies. This work paves the way toward a deeper understanding of how the m^{6}A writer complex functions.

Poster 3

Puf3 contributes to changes in mRNA solubility, translation elongation dynamics at rare arginine codons and loss of protein homeostasis in cells lacking Not4

AUDEBERT, Léna (1), ALLEN, George E. (1), CHEN, Siyu (1,2), PANASENKO, Olesya O. (1,5), HUGH, Suzanne (3), POLTE, Christine (4), IGNATOVA, Zoya (4), PELECHANO, Vicent (3), COLLART, Martine A. (1)

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2: Department of Infectious Diseases, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China;
3: Karolinska Institute, Department of Microbiology, Tumor and Cell Biology, Sweden;
4: Biochemistry and Molecular Biology, University of Hamburg, Germany;
5: The BioCode: RNA to Proteins Core Facility, Faculty of Medicine, University of Geneva

The Not proteins of the Ccr4-Not complex regulate translation elongation dynamics, essential for proper folding and assembly of new proteins. In yeast, ribosomes with non-optimal codons in the A-site are enriched within the pool of ribosomes bound by Not4 and Not5. Such ribosomes accumulate in cells lacking Not4 or Not5, that show defects in co-translational assembly and aggregation of new proteins. Recently we observed that depletion of Not1 and Not4 inversely regulate changes in mRNA solubility, correlating with inverse codon-specific changes in A-site ribosome dwelling occupancies (RDOs). Here we describe that mRNAs less soluble upon Not4 depletion are enriched for targets of the RNA-binding protein Puf3. We determine that Puf3 contributes to inverse changes of A-site RDOs upon Not1 and Not4 depletion, in particular at rare arginine codons, and it contributes to changes in mRNA solubility in absence of not4. Moreover, deletion of Puf3 suppresses temperature sensitivity and protein aggregation in absence of not4, while overexpression of Puf3 is toxic. Puf3 post-translational modifications and the Puf3 interactome are altered in absence of not4. Taken together, our results associate alterations in Puf3 post-translational status and function, including contribution to translation elongation dynamics, with not4 deletion mutant phenotypes.

Poster 4

Reconstitution of human translation elongation complexes

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The ribosome is a large ribonucleoprotein complex that translates proteins from mRNA templates. The fundamental process of peptide bond formation is conserved across all domains of life. The different stages of translation are: initiation, elongation, termination and recycling during which translation factors bind and dissociate from the ribosome and modulate its functional specificity during protein synthesis. Transfer RNAs play an important role in decoding the genetic code. It forms a link between the mRNA at the decoding center of the small subunit and the amino acids attached at the CCA end of the tRNA in the peptidyl transferase center of the large subunit. My project aims to better understand the fidelity mechanisms during translation elongation. For this, I aim to reconstitute human translation elongation complexes and study their structure by cryo-electron microscopy. I have adapted the established protocol to purify 40S and 60S ribosomal subunits and the quality of the sample was analysed by negative staining. I also expressed and purified two aminoacyl tRNA synthetases, and also purified tRNAs. Future works include the purification of elongation factors, aminoacylation of tRNA, complex reconstitution and cryo-EM for structure analysis. Through my research work I'll be able to contribute towards better understanding the molecular mechanisms of translation elongation and how translation fidelity is ensured. Faithful translation of mRNA to protein is necessary to produce functional proteins and maintain cellular homeostasis. Errors like misincorporation events and frameshifting can produce non-functional proteins and disrupt cellular pathways resulting in cancer and other neurodegenerative diseases.

Poster 5

Morpholino-modified ribozymes: Towards cell-stable catalytic RNAs

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Catalytic RNAs present promising applications in studying RNA functions in living cells, enabling precise gene regulation, RNA structural analysis, and exploring the roles of non-coding RNAs in cellular processes. They hold therapeutic potential due to their ability to cleave target RNAs in a sequence-specific manner. However, their application is limited by the inherent instability of ribozymes in cellular environment due to rapid degradation by nucleases. As a step toward developing cell-stable ribozymes, we are investigating strategic incorporation of third-generation antisense oligonucleotide modifications, such as morpholino units, into ribozymes to enhance resistance to nuclease degradation.
We have optimized a synthetic method involving phosphoramidite chemistry [1,2] to incorporate morpholino units into the RNA backbone, generating chimeric oligonucleotides comprising morpholino and RNA. Using this optimized method, we achieved site-specific chemical incorporation of single or multiple morpholino residues into ribozyme binding arms with different P(V) backbone chemistries. As a test case, we used an in vitro selected methyltransferase ribozyme (MTR1), recently engineered in our lab [3,4].
To evaluate the effect of morpholino residues incorporation on substrate binding, we performed UV thermal denaturation studies of duplexes formed by a MTR1 substrate and morpholino-modified binding arms. Moreover, MTR1 with morpholino-modified binding arms has demonstrated successful alkyltransferase activity.
These findings lay the groundwork for rational design of morpholino-modified, cell-stable ribozymes. The next step is rational incorporation of morpholino residues into the catalytic core without affecting catalytic activity. This ongoing work foresees a viable strategy for engineering nuclease-resistant catalytic RNAs with morpholino modifications, paving the way for next-generation cell-stable ribozymes.

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Poster 6

From protein-repeat RNA interactions to novel treatments of C9-ALS/FTD and Myotonic Dystrophy 1

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of the motor system [1]. Frontotemporal dementia (FTD) is characterized by behavioural changes and aphasia [2]. The primary genetic cause of these diseases is the (G4C2) hexanucleotide repeat expansion (HRE) in the first intron of the *C9ORF72* gene, with more than 30 repeats considered pathological [3]. Serine-arginine–rich splicing factor 1 (SRSF1), in complex with NXF1, exports HRE RNAs from the nucleus into the cytoplasm, where they are translated into toxic peptides, regarded as the main cause of C9-ALS/FTD [4]. HRE RNAs can also condense into nuclear foci in neurons [5], and SRSF1 co-localizes with these foci [4]. Here, we unravel the interaction mode of unphosphorylated and phosphorylated SRSF1 with pathological $(G4C2)_{43}$. Based on this, we rationally developed a peptide to inhibit SRSF1 binding to (G4C2)n RNA. Additionally, we identified a potential small-molecule inhibitor of the SRSF1–HRE RNA interaction using ^{19}F NMR screening.
Myotonic dystrophy type 1 (DM1) is characterized by slow muscle relaxation, weakness, and wasting. The disease is caused by a (CTG) microsatellite repeat expansion in the 3′UTR of the *DMPK* gene, with more than 50 repeats being pathological [6]. Sequestration of Muscleblind-like 1 (MBNL1) by (CUG) repeat RNA nuclear foci triggers the disease via MBNL1 loss of function [7]. Here, we investigated the mechanism of MBNL1 phase separation with (CUG)n repeats and performed a FRET-based screen that identified an RNA-binding compound preventing MBNL1 interaction with RNA repeats, thereby disrupting MBNL1–(CUG)n phase separation.

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Poster 7

Dimerization of ADAR1 modulates site-specificity of RNA editing

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Adenosine-to-inosine editing is catalyzed by adenosine deaminases acting on RNA (ADARs) in double-stranded RNA (dsRNA) regions. Although three ADARs exist in mammals, ADAR1 is responsible for the vast majority of the editing events and acts on thousands of sites in the human transcriptome. ADAR1 has been proposed to form a stable homodimer and dimerization is suggested to be important for editing activity. In the absence of a structural basis for the dimerization of ADAR1, and without a way to prevent dimer formation, the effect of dimerization on enzyme activity or site specificity has remained elusive. Here, we report on the structural analysis of the third double-stranded RNA-binding domain of ADAR1 (dsRBD3), which reveals stable dimer formation through a large inter-domain interface. Exploiting these structural insights, we engineered an interface-mutant disrupting ADAR1-dsRBD3 dimerization. Notably, dimerization disruption did not abrogate ADAR1 editing activity but intricately affected editing efficiency at selected sites. This suggests a complex role for dimerization in the selection of editing sites by ADARs, and makes dimerization a potential target for modulating ADAR1 editing activity in the context of immunotherapy.

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Poster 8

Development of a live single-molecule imaging method to visualize IRE1 oligomerization during mRNA splicing and decay in the UPR

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The unfolded protein response (UPR) comprises signaling pathways that monitor protein folding homeostasis in the endoplasmic reticulum (ER) and adjust its folding capacity. The most conserved UPR branch is activated by the ER transmembrane kinase/ribonuclease IRE1α. Upon activation, IRE1 non-canonically splices X-box binding protein 1 (XBP1) mRNA, which then encodes a transcription factor that induces stress-response genes. In addition, IRE1 cleaves a range of mRNAs through regulated IRE1-dependent decay (RIDD). However, the molecular mechanisms that regulate IRE1's catalytic activity, particularly those determining its substrate specificity, remain unclear.

We hypothesize that IRE1 activity could be dynamically regulated through the formation of distinct oligomeric states that modulate cleavage specificity. To investigate these precise oligomeric states during ER stress, we aim to develop a single-protein imaging approach, extending our previously established live single-mRNA imaging method [1] to enable visualization of individual IRE1 molecules together with their mRNA targets.

For extended detection of IRE1 oligomers at high temporal resolution in living cells, we employ a signal amplification strategy using a "spaghetti monster" (SM) tag featuring multiple antigen repeats on solvent-accessible loops. Co-expression of single-chain antibodies (scAbs) against these repeats, fused to fluorescent proteins, enables detection and long-term tracking of individual SM-IRE1 fusion proteins. This method will allow direct quantification of the oligomeric states of IRE1 assemblies that co-localize with different mRNA targets during the UPR.

Here, we present proof-of-principle experiments demonstrating robust detection of SM-tagged ER membrane proteins through recruitment of fluorescent scAbs, which will serve as calibration for the analysis of IRE1 oligomerization dynamics.

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Poster 9

LIN28 inhibition by small molecule reverses miRNA expression and disease hallmarks in myotonic dystrophy

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Sequestration of MBNL1 by expanded CUG or CCUG repeats causes myotonic dystrophy (DM) [1-3]. The pathology of the disease involves dysregulation of ion channels caused by mis-splicing due to MBNL1 sequestration or loss of target miRNAs controlled by RNA binding protein (RBP) [4-7]. It has been shown that disruption of MEF2 transcription network alters miRNA expression in both human and mouse DM1 model and heart samples; an overexpression of MEF2C restores MEF2A target miRNAs in a DM1 cell culture model [8]. Several groups are investigating peptides or small molecule inhibitors of the MBNL1.CUGexp interaction as potential therapeutic approaches for DM [9-10]. We are investigating an alternative approach by inhibition of LIN28 in DM and a small molecule approach to rescue reduced MEF2A expression and dysregulated ion channels in DM model. We have shown that a subset of primary and mature miRNAs which are controlled by either LIN28 or MEF2A are dysregulated in skeletal myotubes derived from DM1 and DM2 patient samples compared to healthy volunteers. We have shown MEF2A expression is depleted whereas LIN28 levels are elevated in DM patient samples. We used small molecule C1632 to inhibit LIN28 and de-repress MEF2A in myotubes from DM samples. We have further shown that C1632 treatment increased the levels of several of these altered miRNAs, and reduced the expression of KCNJ2, CACNA1S, ATP1B1 ion channels, which are involved in DM pathology. Our finding reveals LIN28 inhibition activity could be used as an RNA therapeutics strategy towards pathophysiology of DM or cardiac defects.

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Poster 10

Ψ-Spinach: a light-up aptamer functional with total replacement of uridine by N^1-methylpseudouridine

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With the Covid19 pandemic, modified mRNA-based therapeutics were brought to the forefront and are now extensively developed toward treatment of all type of disease. The direct visualization of these RNA can be useful to improve their pharmacokinetics by understanding how do they enter the cells and where they go after transfection. To do that, fluorogenic light-up aptamers, single-stranded RNA that can bind and activate the fluorescence capabilities of a fluorogen, are promising tools as they can directly be embedded and genetically encoded within the mRNA thus reducing the cost of production and bypassing the need of cells overexpressing fluorescent cofactors. However, to be safer and more effective, these mRNA therapeutics often rely on the complete substitution of uridine by N^1-methylpseudouridine, and no light-up aptamer has been described so far with this modification. In this poster, I will present the *in vitro* evaluation of various fluorogenic light-up aptamers showing that most of them are not functional with the modification, emphasizing the need to develop new one. The presented work will then cover such a development by describing the selection and characterization of Ψ-Spinach, a new RNA fluorogenic light-up aptamer efficient with N^1-methylpseudouridine, developed by directed evolution combined with microfluidic-assisted *in vitro* compartmentalization.

Poster 11

The role of NUDIX enzymes in cellular metabolism and non-canonical mRNA capping

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RNA polymerase II can use nucleoside metabolites and cofactors as initiating nucleotides, resulting in metabolite-capped RNAs. These non-canonical mRNA caps may provide a novel mechanism for regulating gene expression, linking cellular metabolism directly to transcription. In vitro, such non-canonical caps can be cleaved by enzymes belonging to the nucleoside diphosphate linked to another moiety X (NUDIX) family. However, the in vivo substrates of most of the 22 human NUDIX enzymes remain unclear, as do their biological functions. To elucidate the physiological roles of these enzymes, we used a comprehensive library of 18 NUDIX knockout cell lines. First, we observed growth defects in all mutants with increased apoptosis and cell cycle arrests. Second, we found alterations in cell morphology and organelles, including mitochondria and P-bodies. Furthermore, many mutants exhibited abnormal nuclear shapes, micronuclei, and increased DNA damage. Third, gene expression differed significantly between the mutants and the wild type. Interestingly, knocking out any NUDIX enzyme affected the expression levels of other NUDIX enzymes, suggesting the presence of compensatory mechanisms. Additionally, we observed increased innate immune responses in several mutants in different cell lines, consistent with previous reports that non-canonically capped RNAs can trigger the innate immune system. Finally, metabolomic profiling revealed alterations in the purine biosynthetic pathway in all mutants. Our results underscore the physiological relevance of NUDIX enzymes; however, further in vivo analyses and integration of multiomics data from all mutants are needed to better understand their functions and interplay with non-canonical mRNA caps.

Poster 12

Harnessing epitranscriptomics to detect and manage pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) remains the deadliest cancer, mainly due to late diagnosis and limited treatments. The identification of early-detection biomarkers could therefore significantly improve patient outcomes. Recently, RNA modifications (epitranscriptomics) have emerged as an additional regulatory layer of gene expression in both healthy tissues and cancer, controlling the post-transcriptional fate of RNA in response to microenvironmental changes and stress. Modified nucleosides released during RNA turnover can be detected in blood, and recent studies suggests they may bind cell-surface receptors and modulate immune and inflammatory signalling. We hypothesize that profiling circulating modified nucleosides could reflect early carcinogenic alterations and contribute to tumor development.
My PhD aims to establish a circulating chemical signature for early PDAC detection, characterise RNA modification dysregulation within the tumour microenvironment, and explore the signalling potential of circulating nucleosides.
Plasma samples from PDAC patients (n=32) and healthy donors (n=20) were used to extract free nucleosides (FN), using methanol. FN epitranscriptomic profiles were determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). In parallel, the capacity of nucleosides to bind and activate A2A adenosine receptors was assessed in CHO-A2A cells by monitoring cAMP production
We established a circulating "epitranscriptomic signature" of 6 nucleosides significantly associated with the presence of PDAC showing promising diagnostic performance (100% specificity, 90.5% sensitivity). However, validation in a larger cohort is still required. Mechanistically, our preliminary results suggest that circulating modified nucleosides are not mere degradation byproducts but may also function as signalling molecules via adenosine receptors, potentially influencing inflammation and immune responses.

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Poster 13

Evolution of dosage compensation mechanisms in insects

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In many animals, sex is determined genetically by sex chromosomes, most commonly the XX–XY system, where females carry two X chromosomes and males one X and one Y. However, the unequal number of X chromosomes between the sexes creates a fundamental problem of gene dosage imbalance, which is resolved through diverse dosage compensation (DC) strategies. Well-studied model organisms illustrate different solutions: human females inactivate one X chromosome, hermaphrodites of the nematode *Caenorhabditis elegans* partially dampen expression from both X chromosomes, and male fruit flies (*Drosophila*) and mosquitoes (*Anopheles*) boost the expression from their single X chromosome to match female expression. Notably, while *Drosophila* promotes the transcription of X-linked genes via deposition of the activating histone mark H4K16ac [1,2], *Anopheles* relies on an H4K16ac-independent mechanism involving the species-specific gene SOA [3].
Recent advances in genome sequencing have revealed that the Diptera order, where both *Drosophila* and *Anopheles* belong, turns out to be an exception among insects; most other insect orders carry a distinct, independently-derived and deeply conserved ancestral X chromosome that has persisted for over 450 million years, making it the oldest known sex chromosome [4]. The molecular mechanism of DC in species with the ancient X chromosome remains unknown. My project aims to fill this knowledge gap by elucidating the molecular mechanism of DC across insect species from ten orders, focusing on the Bacillus stick insects for further characterization. Preliminary data indicate that neither H4K16ac nor a SOA homolog is involved, suggesting the presence of a distinct, yet-to-be-described compensation mechanism.

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Poster 14

Immunopeptidome and proteome responses to SMG-1–mediated NMD inhibition in NSCLC show a potential transition toward a hot tumor phenotype

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Tumors are classified as cold or hot based on their immune infiltration, with cold tumors exhibiting low or absent immune cell presence and reduced visibility to the immune system. In contrast, hot tumors are highly infiltrated by cytotoxic CD8+ T-cells and other immune effectors, often showing inflammation and immune response activation. Inhibition of nonsense-mediated mRNA decay (NMD) has been shown to increase tumor antigenicity and is proposed as a strategy to shift cold tumors toward a more immunogenic, hot phenotype. Our goal is to achieve a broader understanding of the antigen presentation landscape upon NMD inhibition using the SMG-1 inhibitor 11e. For this, we established a small-scale workflow capable of isolating, identifying, and characterizing hundreds of HLA class I-presented peptides from fewer than 10^7 cells. This approach employs an in-house functionalized resin capturing HLA-A, -B and -C complexes, followed by mass spectrometry analysis of associated peptides. Using non–small cell lung cancer (NSCLC) cells treated with the SMG-1 inhibitor 11e, we are investigating the neoantigen landscape of treated versus untreated conditions to assess whether NMD inhibition alters the presentation of tumor-associated or potentially neoantigenic peptides in our model system. In parallel, whole-cell proteomics and phosphoproteomics under the same conditions will provide insight into the molecular consequences of 11e treatment and help contextualize immunopeptidome changes.

Poster 15

N^6-methyladenosine (m^6A)-mediated translation control of DGKk by FMRP governs diacylglycerol-dependent neuronal translation and activity

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Fragile X syndrome (FXS) is a leading cause of inherited intellectual disability and autism, resulting from the absence of the RNA-binding protein FMRP. FMRP deficiency leads to aberrant neuronal protein synthesis associated with neurological alterations. We previously showed that diacylglycerol kinase kappa (DGKκ) is a primary mRNA target of FMRP in cortical neurons [1]. DGKκ regulates diacylglycerol signaling and its downregulation due to FMRP loss could account for several main alterations observed in FXS [1]. Moreover, re-expressing an FMRP-independent form of DGKk via adeno-associated viral (AAV) vector in the brain of Fmr1-KO mice provided multiscale long-term correction of the phenotypes [2].
In this study, we demonstrate that Dgkk-KO mice exhibit phenotypes similar to Fmr1-KO mouse, including an excessive neuronal translation rate which supports the hypothesis that many altered proteins in FXS are due to altered DAG signaling. These results highlight the central role of DGKk in neuronal signaling related to protein synthesis and neurological functions.
Furthermore, we show that FMRP specifically binds to DGKk mRNA throughout the brain, and positively modulates its translation.This regulation occurs through FMRP's interaction with repeated motifs that are highly modified with N^6-methyladenine (m^6A). We identify a mechanism wherein FMRP helps alleviate a translational blockade in an m^6A dependent manner. Overall, this study demonstrates the pivotal role of DGKκ in brain functions and its contributions to FXS, while proposing a novel model by which FMRP controls the expression of a key mRNA target.

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Poster 16

The RNA methyl-transferase enzyme FTSJ1: conserved role in neuron morphology & learning performance

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Transfer RNAs (tRNAs) are crucial for translation, with their function heavily influenced by modified ribonucleotides. One such modification, 2'-O-methylation (Nm), affects the ribose moiety and is particularly present on the anticodon loop of some tRNAs. In humans, Nm is catalyzed by the SAM-dependent methyltransferase FTSJ1. Loss of FTSJ1 leads to intellectual disability (ID), though the mechanisms are not fully understood. Our studies in human neural progenitor cells showed that inhibiting FTSJ1 increases dendritic spines, a feature common in neurodevelopmental disorders. This phenotype is also observed in Drosophila larvae with mutated FTSJ1 orthologs and mice. Transcriptome analysis revealed deregulation of mRNA and miRNA involved in brain morphogenesis in human cells, suggesting defective gene expression regulation contributes to the observed morphological defects. Additionally, long-term memory is affected in Drosophila mutants of FTSJ1. Given tRNAs' role in translation, transcriptome-wide profiling of ribosome footprints was conducted on human and Drosophila cells affected by FTSJ1 activity. These analyses are ongoing and recent results will be presented. Those results indicate significant regulation of brain-specific genes, morphological defects in neuronal cells lacking FTSJ1 and codon bias. The goal is to identify genes involved in the defective morphology of neuronal tissues without tRNA Nm, determine if the regulation occurs at the translational level, and understand the mechanisms behind FTSJ1-related intellectual disability.

Poster 17

When neuronal box C/D snoRNAs meet genomic Imprinting: Insights into Prader-Willi Syndrome

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Box C/D small nucleolar RNAs (SNORDs) guide site-specific 2′-O-ribose methylation of rRNA, U-snRNAs and tRNAs, and, to a lesser extent, direct N4-acetylcytidine formation in rRNA and contribute to pre-rRNA processing. For many SNORDs, however, their molecular functions remain unclear because no validated target RNAs have been identified. Notably, the expression of numerous “orphan” SNORDs is governed by genomic imprinting, an epigenetic mechanism that silences one parental allele in a parent-of-origin-specific manner. These imprinted SNORDs are often arranged in large tandem arrays containing dozens to hundreds of related copies.
Particular attention has focused on the paternally expressed SNORD64, SNORD107, SNORD108, SNORD109A/B, SNORD115 and SNORD116 genes, embedded within a large imprinted chromosomal domain at human15q11-q13. This region is genetically linked to Prader-Willi syndrome (PWS), a neurodevelopmental disorder primarily affecting hypothalamic function. This rare disease is associated with a broad spectrum of developmental, metabolic, behavioral and endocrine abnormalities. PWS typically presents with perinatal hypotonia and poor feeding, followed in early childhood by hyperphagia that can lead to severe obesity if untreated. Although genotype-phenotype relationships remain incompletely resolved, clinical evidence highlights the importance of paternally inherited deletions spanning the SNORD115-SNORD116 interval, with the SNORD116 array emerging as a key contributor.
Using SNORD115-KO, SNORD116-KO and SNORD115/116 double-KO mouse models (unpublished), I aim to summarize current knowledge of the molecular, physiological and evolutionary roles of these atypical, imprinted small regulatory RNAs in brain function, and to outline their potential contributions to PWS etiology.

Poster 18

A study of RNA as a molecular memory system: new perspectives from long-lived RNAs

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Cells and organisms must store molecular information across time in a stable yet flexible manner to differentiate, adapt and potentially transmit information about life experiences. While DNA stores information and has long-term genomic stability, its fixed sequence cannot explain dynamic or heritable changes induced by environmental exposures. RNA, traditionally viewed as a transient messenger, is increasingly recognized for its regulatory roles and its ability to mediate activity-dependent dynamic responses. Recently, unexpectedly stable RNA populations called long-lived RNAs (LL-RNAs), were discovered in the mouse brain, where they persist across life and contribute to chromatin organization [1]. This raises the possibility that RNA may serve as a molecular memory substrate that could ultimately contribute to stable and heritable changes. This project builds on this concept and aims to study the stability of RNA in the germline, the cellular substrate for heredity. Our preliminary results show that RNA molecules can persist for over a month in specific mouse testis cells, suggesting the existence of LL-RNAs in the germline. Based on these results, this project will establish a spatiotemporal atlas of germline LL-RNAs, assess how their profiles are modulated by environmental exposures such as early-life stress and enrichment, and test their contribution to the transfer of molecular information from father to the offspring. Using RNA metabolic labeling, sequencing, imaging and functional perturbations both in vivo and in vitro, it will investigate how LL-RNAs are maintained and regulated, what roles they play, and whether and how they are involved in transmitting information across generations.

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Poster 19

Gene specific splicing correction with small molecules: fundamental insights into gene selectivity and rational design

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Small molecule splicing modifiers represent an innovative therapeutic modality able to correct splicing in a gene-specific manner. Their relevance emerged during the development of an oral treatment for Spinal Muscular Atrophy [1]. In 2019, the mechanism of action of the prototype SMN-C5 was elucidated leading to the discovery of 5'-splice site bulge repair [2]. SMN-C5 acts as a molecular glue at the interface between the U1 snRNP and the SMN2 exon 7 5'-splice site by stabilizing the unpaired A-1. SMN-C5 allosterically promotes the exon 7 inclusion in the SMN2 mRNA, restoring the functional SMN protein production in SMA patients [2,3]. Optimization of SMN-C5 enabled the discovery of Risdiplam, later FDA-approved as an orally available SMA treatment. Furthermore, other splicing modifiers investigated by NMR spectroscopy revealed shared features [3]. Importantly, the activity of these small molecules also depends on a purine-rich element upstream of the 5′-splice site, suggesting that specificity arises from recognition of a larger quaternary structure involved in 5'-splice site repair mechanism [4]. To better understand the gene selectivity of SMN2 splicing modulators, our strategy is to isolate the splicing correction complex assembled around Risdiplam on SMN2 exon 7 and to determine its composition and its structure. Additionally, our project aims to discover novel splicing modifiers acting on C-1 and U-1 bulged 5'-splice site. We launched an in silico screening campaign using small molecules databases and potential compounds emerged. Their structural specificity is analysed through NMR spectroscopy while cell-based assays are performed to evaluate their biological activity.

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Poster 20

Lucid platform: A Lab Unified Computational Integration and Discovery platform for data management and enhanced collaboration

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Biology laboratories working on RNA and related areas accumulate years of heterogeneous, largely unstructured data, such as count tables, images, theses, publications, ELN-like notes and other assay outputs, that are difficult to search, reuse and explain to new lab members. We present LUCID, a fully local, secure platform that organises historical and ongoing lab data into reusable, queryable “structured data packages” to enhance discovery, onboarding and collaboration, even without internet access.
Diverse assets, including newly generated results and public or synthetic multi-omics datasets, are ingested and mapped into a compact schema (projects, experiments, samples, runs, qRT-PCR results, DEG results) plus a searchable document index. Together, these form an evolving “lab memory” rather than a collection of static files.
On top of this schema, a lab-focused agent backed by a private large language model (LLM) accepts natural-language questions and decomposes them into structured queries and document retrieval. Queries such as “Where did we investigate LINE-1 expression in RNAi mutant cells?” return relevant experiments, associated samples and quantitative readouts, linked theses and papers, and short summaries grounded in those sources, with preserved provenance. Interactions with the agent (questions, answers and linked datasets) can be stored as shared threads that lab members revisit, extend and comment on asynchronously, fostering collaboration around specific topics or datasets.
We illustrate the approach using a Ciaudo-lab-inspired RNA use case with public and synthetic data. The schema-centred design is intended to be reusable across laboratories with similar data characteristics, enabling collaborative use of mixed structured and unstructured information.

Poster 21

Studying protein-RNA interactions? The NMR platform can help you out!

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The Biomolecular NMR Spectroscopy Platform (BNSP) of the Department of Biology at the ETH Zürich offers expertise and infrastructure for the study of biomolecules in solution. We give here an overview of the platform and report on some of the recent data obtained in the context of the NCCR "RNA & disease".
In collaboration with the Robinson and Panse's labs, we published a manuscript on the characterization of Npl3 interaction with RNA suggesting an unanticipated RNA chaperoning role for Npl3 during spliceosome active site formation [1]. With the Hall's lab, we investigated the interaction of Lin28 with different RNA-PROTAC molecules [2] and the effect of inosine substitutions in G-quadruplexes [3]. We also participated to a structural investigation of Cas9 off-target activity in collaboration with the Jinek's lab [4] using SwitchSense. This technology allows to measure the motion of biomolecules on a chip and to capture ultra-fast and ultra-slow kinetics. Kd values can be measured from the fM to mM using low concentrations of unlabeled molecules. This approach can be used to measure differences in affinity between WT and mutated versions of protein-RNA complexes and can be extended to the study of protein-DNA, protein-protein and protein-small molecules interactions. We are also working on two collaborative projects with the Polymenidou's lab on TDP-43 [5]. Finally, we recently started a collaboration with the Polacek's lab to characterize the interaction of CsrA with the Fim R2 RNA using NMR.

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Poster 22

Investigating the kinetic difference in translation termination at stop codons of NMD-sensitive versus NMD-insensitive transcripts

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Previous studies in the lab examined whether there is a kinetic difference in translation termination between NMD-eliciting stop codons and regular stop codons, both in vivo and in vitro. In vivo, a monosome profiling (Ribo-seq) of HeLa cells was used, and ribosome occupancy at stop codons was determined. In this study, no clear differences were found between the ribosome occupancy at NMD-stimulating stop codons and regular stop codons [1]. However, because of limited sensitivity and technical concerns, putative moderate kinetic differences in translation termination might have been missed.
Hence, I will revisit this central question of substrate recognition in NMD using disome profiling as an alternative approach. We might have missed existing kinetic differences in the monosome profiling because there is no reliable method to retain the ribosomes at stop codons after cell lysis. Since slowed-down termination is expected to increase ribosome collisions upstream of a stop codon, I plan to perform disome profiling in HeLa cells. Unlike terminating ribosomes, these collided ribosomes are still in the elongation mode and can be stabilized with cycloheximide. We expect disome profiling to be more sensitive and reliable for identifying putative kinetic differences in translation termination. The results from disome profiling will be intersected with our list of high-confidence NMD targets in the same HeLa cells to determine whether slower translation termination is indeed a hallmark of NMD-sensitive transcripts, or even the NMD-triggering feature.

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Poster 23

Metabolic regulation via m^{6}Am cap modification of mammalian mRNAs

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The epitranscriptome represents an additional layer of gene regulation, influencing RNA life processes such as RNA processing, stability, translation, and localization. Eukaryotic mRNAs are capped at their 5' end with a modified guanosine nucleotide, usually referred to as the m^{7}G canonical cap structure, which is essential for mRNA life from yeast to plants and human. While many higher eukaryotes incorporate ribose methylation (N_m) on the first transcribed nucleotide, mammals and other vertebrates introduce an additional modification on the first nucleotide: *N*^{6}-methyladenosine (m^{6}A$_m$), catalyzed by the cap-specific methyltransferase PCIF1. Despite the prevalence of m^{6}A$_m$, *Pcif1*-depleted mice exhibited normal development and fertility. However, when challenged with a high-fat diet *Pcif1* mutant mice failed to gain as much weight as the control mice. I will present our study that links the m^{6}A$_m$ cap modification to control of body weight through regulation of fat deposits in mice.
Far from being a static fixture on eukaryotic mRNAs, these findings highlight a potential role for the canonical cap modification in metabolic gene regulation, expanding our understanding of the cap modification's role in mammalian physiology.

Poster 24

Decoding recruitment: linking splicing arrest to mRNP accumulation in nuclear speckles

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Nuclear speckles are prominent biomolecular condensates that have emerged as regulatory hubs for gene expression. Recent studies show that nuclear speckles localize near highly transcribed genes and enhance splicing efficiency. However, the molecular mechanisms governing mRNA recruitment to nuclear speckles and its impact on mRNA processing remain largely unknown.
We combine targeted perturbation of spliceosome progression with quantitative imaging and proteomics to define a splicing-coupled checkpoint that routes incompletely spliced mRNPs to nuclear speckles and prevents their premature export.
A systematic screen of ATPase-deficient mutants of all DDX/DHX helicases associated with nuclear speckles reveals that arrests early in the splicing cycle, mediated by DDX23, DDX42, and DHX16, trigger pronounced nuclear speckle enlargement and robust poly(A) RNA accumulation. Affinity purification of these stalled mRNPs followed by mass spectrometry identifies a set of 'recruiter' proteins that become enriched on arrested mRNPs. Recruiter candidates relocalize from the nucleoplasm to nuclear speckles upon splicing inhibition, and their depletion markedly reduces nuclear speckle enlargement and poly(A) RNA accumulation in untreated and splicing-inhibited cells.
Using super-resolution microscopy, we find that nuclear speckles are not homogeneous condensates. Instead, their core components SON and SRRM2 form meshwork-like scaffolds that remodel upon transcription or splicing inhibition. Depletion of recruiter candidates results in smaller speckles with perturbed meshwork architecture, suggesting that nuclear speckle ultrastructure is highly dynamic and shaped by mRNP recruitment.
Together, these results identify factors required for mRNP retention in nuclear speckles and provide new clues about how nuclear speckle morphology is coupled to mRNP splicing and maturation.

Poster 25

Investigating the interactions of MDA5 and LGP2 with self-RNA

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MDA5 and LGP2 are proteins from the Rig-I-Like-Receptor (RLR) family, a group of cytoplasmic pattern recognition receptors (PRR) that sense double-stranded (ds)RNA and initiate an immune response upon certain viral infections. This mechanism serves as protection against viral infections, but when dysregulated may result in autoimmune diseases. MDA5 forms filaments along dsRNA to activate a protein on the mitochondrial surface and induce type I interferon signaling, while LGP2, though unable to bind the mitochondrial protein itself, enhances MDA5 mediated signaling. LGP2 is also required for interferon responses in cells lacking ADAR1, an enzyme that edits endogenous dsRNA through Adenosine to Inosine. This editing alters dsRNA structure, preventing its recognition by MDA5 and thereby disrupts signaling. How exactly these seemingly small alterations in the dsRNA structure affect the binding dynamics of MDA5 and LGP2 remains unknown. This mechanism is highly interesting, because endogenous MDA5 ligands themselves contain mismatches and bulges, even if they are not edited by ADAR1. How these inherent structures influence RLR binding is not fully understood. Therefore, we aim to investigate how introduction of structural elements into duplex RNA affects the binding and translocation of LGP2 and MDA5 on the dsRNA. In order to answer this question, we are establishing a Total Internal Reflection Fluorescence (TIRF) Microscopy experiment, that allows us to measure single MDA5 or LGP2 molecules that bind the dsRNA and track their trajectories along the RNA. By introducing known structural changes into the dsRNA, we will be able to deduce effects on MDA5 and LGP2.

Poster 26

RNA orchestrates hnRNP condensation and directs 40S hnRNP ribonucleosome assembly

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The 40S hnRNP ribonucleosome is considered the primary pre-mRNA packaging unit and is mainly composed of HNRNPA and HNRNPC proteins [1-3]. Despite the studies done in individual components of the complex, the mode of interaction between them and the mechanism driving particle assembly remains elusive. HnRNPs high cell concentration and long disordered regions, make them prone to phase-separation (PS). Combining biochemical and cell biological approaches, we showed that HNRNPC1 undergoes self-PS at physiological concentration, mediated by weak interactions involving aromatic and charged residues. HNRNPA1 is recruited via its disordered region to form co-condensates, that RNA can further join enhancing PS. Further RNA addition rigidifies and restructures these condensates, increasing internal protein concentration and ultimately leading to 40S hnRNP particles formation and condensation dissolution. Mutagenesis of HNRNPC1 PS-driving residues affects the 40S ribonucleosome assembly and its function in splicing. Unlike its DNA counterpart - the nucleosome, a soluble particle that undergoes PS to increase compaction-, the ribonucleosome originates from condensates and folds into complexes upon RNA binding.

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Poster 27

Bacterial 70S Scanning - An overlooked yet crucial regulatory mechanism for translation initiation?

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Initiation, the process of assembling the ribosomal subunits at the correct start site on the mRNA, is both the first and the rate-limiting step of translation, embodying enormous regulatory potential. Accordingly, canonical textbook initiation has been studied extensively. Nevertheless, alternative forms of initiation have been described.
One neglected alternative path of initiation in bacteria is 70S scanning, also known as 70S re-initiation.
During 70S scanning, the ribosome translates at least two genes on a polycistronic transcript without disassociating the subunits after translation of the first gene. Instead, the intact 70S complex scans along the mRNA and re-initiates at the downstream gene.
Two standalone studies have shown that 70S scanning is feasible [1,2], but a detailed understanding is lacking. Surprisingly, a recent study suggested that over half of the 70S complexes in *E. coli* do in fact re-initiate without splitting [3]. Considering the high number of polycistronic mRNAs in bacteria, these observations are indicative of the severely underestimated importance of 70S scanning in protein synthesis, potentially representing a novel important layer of translation regulation.
Therefore, with the goal to expand our understanding of one of the most fundamental processes in bacterial life, this project aims to unravel for the first time the underlying mechanistical details of 70S scanning. Furthermore, we aim to spearhead future research of this alternative path of initiation with a special focus on its regulatory potential for medically relevant conditions such as its contribution to antibiotic resistances.

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Poster 28

Molecular Architecture of an Endogenous Mouse PIWI protein

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PIWI proteins and their bound 24-30 nt PIWI-interacting RNAs (piRNAs) are essential for transposon control [1-3]. The small RNA acts as a guide to identify targets via perfect complementarity and direct the endoribonuclease activity of PIWI proteins to destroy transposons to maintain genome integrity. Mouse PIWI protein MIWI is unique in binding sequences called pachytene piRNAs, which lack complementary to any sequence in the transcriptome [4,5]. Nevertheless, a single point mutation in MIWI that renders it catalytically dead, results in mouse sterility, highlighting the critical importance of its target cleavage function [6]. Search for putative targets and definition of targeting rules identified mRNAs as potential targets and partial complementarity mode of engagement with target RNAs [7-12]. Additionally, the cofactor GTSF1 enhances the catalytic activity of MIWI [13-15]. However, how MIWI uses partial complementarity engagement and interacts with activity-enhacing factors, remain poorly understood.
In this study, we present the first endogenous mammalian PIWI structure, by isolating MIWI from mouse testes. It reveals important architectural features required for RNA targeting, explaining how it can support partial complementary interactions. We also provide structural evidence for a putative tryptophan-binding pocket in the catalytic domain of MIWI that may mediate interaction with mammalian GTSF1. These findings provide deeper insights into MIWI's structure-function relationship, enhancing our understanding of its mechanistic role in RNA-guided gene regulation.

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Poster 29

Protein interaction network of human m^{5}C RNA methyltransferases

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The RNA methyltransferase protein family NOL1/NOP2/SUN (NSUN) comprises seven members that methylate cytosine at position 5 in RNA (5-methylcytosine, m^{5}C). They target a diverse array of RNA substrates, including ribosomal RNA (rRNA), transfer RNA (tRNA), other non-coding RNA (ncRNA), as well as messenger RNA (mRNA). While most research focuses on the functional consequences of m^{5}C on RNA, less is known about the NSUN enzymes themselves. In particular, protein-protein interactions of the NSUN family remain understudied, despite their potential involvement in regulating methylation activity, subcellular localization, and possibly other previously unexplored functions of the RNA methyltransferases.
To address this knowledge gap, we investigate protein interactors of NSUN family members using both chromatographic purification and proximity-dependent biotinylation approaches. By identifying interaction partners, we aim to uncover further insights into the mechanisms governing NSUN proteins.

Poster 30

Image-Based Discovery of a Recurrent Tumor Archetype Characterized by Aberrant RNA Splicing and Associated with Poor Survival in Breast Cancer

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Intra-tumor heterogeneity (ITH) is a major driver of cancer progression and therapeutic resistance. Although ITH has been extensively profiled at the molecular level, revealing the coexistence of tumor subpopulations with distinct molecular identities [1-3], how these programs organize spatially within tumors, forming recurrent regions with unique molecular profiles, tissue architecture, and cellular composition, remains poorly understood. In this study, we push the boundaries of histopathology foundation models (hFMs) to reveal molecularly distinct tumor regions that are invisible to human experts, offering new insights into the molecular organization of invasive breast tumors.

We specialized generalist hFMs through extended pre-training on invasive tumor tissue, enhancing their ability to encode richer, tumor-specific biological concepts. Based solely on these image-derived features, the model identified distinct tumor regions, termed tumor archetypes, that subsequently mapped to unique molecular signatures. Characterizing these tumor archetypes by integrating spatial transcriptomics data, we uncovered distinct patterns of RNA splicing dysregulation alongside other pathways such as TGF-β signaling. Notably, aberrant RNA splicing emerged as the main driver of one archetype across both HER2+ breast cancer and triple-negative breast cancer (TNBC).

Strikingly, these archetypes coexist as spatially distinct regions within the same tumors and recur across patients. Survival analysis revealed that enrichment of the RNA splicing–dysregulated archetype is associated with poorer overall survival, highlighting its clinical relevance.

This work provides a scalable method to uncover tumor archetypes from H&E images, linking tissue morphology to RNA programs and opening new avenues for precision oncology and RNA-focused therapeutic stratification.

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Poster 31

Correcting a pathogenic G>A point mutation in SBF1 gene by Site-directed RNA editing

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Adenosine (A) to Inosine (I) RNA editing is a frequent epitranscriptomic alteration in humans, mediated by ADAR enzymes that act on double-stranded RNA (dsRNA). Once formed, inosine is interpreted as guanosine (G) by most cellular machineries. While traditionally studied due to its dysregulation in various human diseases, A-to-I RNA editing is now emerging as a promising strategy for correcting pathogenic G>A point mutations by leveraging endogenous ADARs. In recent years, several programmable RNA editing platforms have been developed that enable the correction of disease-associated mutations at RNA level, without introducing permanent changes into the genome. Within this framework, we employed the LEAPER (Leveraging Endogenous ADAR for Programmable Editing of RNA) technology to correct the Arg763His (CAC>CGC) mutation in the SBF1 gene, which causes Charcot-Marie-Tooth disease type 4B3. To specifically target a plasmid-borne mutated SBF1 transcript in HEK293 cells, we designed a 151 nucleotides plasmid-borne circular guide RNA (circ-gRNA). A moderate degree of correction (~23%) with several bystander editing events was observed, indicating the potential of this approach. Subsequently, multiple guides were designed and tested to improve editing efficiency and minimize bystander events. Although we succeeded in reducing bystander events, no significant increase in on-target A editing was achieved. To reach this goal, further guide optimization using a randomized plasmid library is currently ongoing.

Poster 32

Polysomes and mRNA control the biophysical properties of the eukaryotic cytoplasm

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The organization and biophysical properties of the cytoplasm influence all cellular reactions, including molecular interactions and the mobility of biomolecules. The cytoplasm does not behave like a simple fluid but is a densely crowded and highly organized environment. However, its detailed properties, the molecular mechanisms that control them, and how they influence the cellular biochemistry remain poorly understood. Here, we investigate the diffusive properties of the cytoplasm in silico and in vivo, employing mRNPs (messenger ribonucleoproteins) and GEM (genetically encoded multimeric) particles as rheological probes. Cytoplasmic diffusivity increases upon polysome disassembly or a reduction in mRNA levels. Reducing ribosome concentration by up to 20%–25% without altering polysome levels has no effect in vivo. Furthermore, mRNA condensation into P-bodies upon polysome disassembly does not affect cytosolic diffusion in budding yeast. Our results demonstrate that mRNAs and their organization into polysomes regulate the biophysical properties of the eukaryotic cytoplasm.

Gade et al., 2025, Cell Reports 44, 116204

Poster 33

Investigating ASOs as a non-genetic tool for Lactobacilli

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Antisense oligomers (ASOs) are RNA-based therapeutics that recognize target RNAs in a programmable, sequence-specific manner and can thereby modulate gene expression. In eukaryotic cells, ASOs have been successfully used to modulate RNA splicing and thereby address difficult-to-treat diseases such as spinal muscular atrophy. By specifically targeting mRNAs of essential bacterial genes, ASOs have been shown to kill bacterial pathogens, representing a new class of antibiotics. The commensal *Lactobacillus sp.* is commonly found in the human oral cavity, gut, lung, and female genital tract. Recent studies suggest that certain *Lactobacillus sp.* possess beneficial probiotic effects on inflammatory responses, neurological diseases, and hypertension. However, the underlying molecular mechanisms involved in this host-microbe interplay have received little attention, partially due to the lack of genetic tools for *Lactobacilli*. ASOs could overcome this problem by modulating gene expression and thereby allowing investigation of gene function. Among different ASO modalities, peptide nucleic acids coupled to cell-penetrating peptides as carriers have been successfully used in gram-negative aerobic bacteria. This project aims to use ASOs to modulate gene expression in gram-positive anaerobic bacteria; specifically, *Lactobacilli*. We have designed ASOs against four different essential genes to assess general efficacy through growth inhibition. All of our conjugates showed concentration-dependent growth inhibition. In ongoing experiments, we are trying to establish a cell morphology-based read-out assay for ASO-mediated inhibition. Our ultimate goal is to establish ASOs as a robust tool for modulating *Lactobacilli*. To better understand how ASO treatment affects the bacterium, we will use RNA-seq to monitor global transcriptomic changes.

Poster 34

Tuning lipid nanoparticle coronas through statistical copolymers for cell-specific gene expression

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Lipid nanoparticles (LNPs) are well-established gene delivery systems in pharmaceutical sciences. Poly(ethylene glycol) (PEG) is commonly used to stabilize LNPs by forming a protective hydrophilic "corona" on the outer surface. However, PEG is associated with hypersensitivity reactions and the development of anti-PEG antibodies [1]. In this work, we investigated poly(2-alkyl-2-oxazoline) (POx)–based statistical copolymers as alternative "stealth" materials to overcome current limitations of LNPs and expand their therapeutic applicability [2].
A library of 42 statistical POx copolymers, combining different hydrophilic and hydrophobic repeating units, was synthesized and screened for usage in microfluidic LNP formulation. Two exemplary candidates with different architectures of the hydrophobic anchoring units (linear vs. branched), showing favorable physicochemical properties regarding particle size and polydispersity, were selected for further evaluation. Upscaling production using a commercially available microfluidic device allowed for investigating gene expression over time and across multiple cell types, including immortalized cell lines and cells derived from human induced pluripotent stem cells.
Our results demonstrate that tuning the architecture of the statistical POx copolymer modulates cell- and time-dependent gene expression profiles. Further experiments indicated low toxicity and immunogenicity of our particles, supporting the usage of statistical POx copolymers as promising alternatives to PEG in LNP formulations.

[1] Chen, Y.; Su, Y.-C.; Roffler, S. R. Polyethylene glycol immunogenicity in nanomedicine. Nature Reviews Bioengineering 2025, 3 (9), 742-760.
[2] Weber, C.; Czaplewska J. A.; Schubert U. S.; Klüpfel J.; Steffens E.; Holthoff H.-P.; Ungerer M.; Gutmann M. O.; Gerolimetto G.; Meinel L.; Wang X.; Dirauf M.; Friebe B.; Copolymers and lipid compositions for lipid nanoparticles. Patent No. WO/2025/168810, August 14, 2025.

Poster 35

Sustained miR-802 expression redirects KRAS-driven neoplastic fate toward IPMN-like lesions

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Pancreatic ductal adenocarcinoma (PDAC) frequently arises from two principal precursor lesions: pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasm (IPMN). While PanINs account for the majority of PDAC cases, IPMNs represent approximately 20% and are generally associated with a more favorable clinical outcome. The prevailing model attributes the divergence between these lesions to distinct cells of origin—acinar cells for PanIN and ductal cells for IPMN.
Previously, our lab identified the microRNA miR-802 as an important regulator of PanIN initiation. Upon mutation KrasG12D, Mir802 expression is diminished, favoring pancreatic cancer oncogenesis. Here, we generated a Ptf1a-Cre; KrasG12D; Mir802-overexpression mouse model capable of counteracting KrasG12D-mediated downregulation. We observed that, contrary to expectations, the sustained expression of Mir802 led to the formation of precancerous lesions that resembled IPMNs. Using the adult-specific Ptf1a-CreER and Sox9-CreER mouse models, respectively for acinar and ductal cells, we failed to recapitulate the IPMN phenotype observed with the non-inducible Ptf1a-Cre, demonstrating that the canonical “two cells, two lesions” paradigm is insufficient to explain IPMN initiation fully. These findings suggest that more plastic cell populations may serve as the source of IPMNs, directing our focus toward pancreatic stem cells. To explore the molecular mechanisms behind lesion fate, we employed laser-capture microdissection followed by RNA sequencing of PanIN and Mir802-derived IPMN lesions. We observed enriched ontologies related to motility and intracellular reorganization, highlighting a critical contribution of miR-802 abundance in directing neoplastic lineage commitment.

Poster 36

The role of RNA modifications in the superwobbling $tRNA^{Val}$ of *M. capricolum*

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Transfer RNAs (tRNAs) are crucial adapter molecules essential for decoding the genetic code during the process of protein synthesis. While they share a characteristic L-shaped structure, their sequences and modifications vary widely. These modifications are located at different positions within the tRNA, thereby influencing the structural stability and decoding.
To study the molecular basis of "superwobbling," we generated unmodified and site-specifically modified variants of the *M. capricolum* $tRNA^{Val}UAC$ via splinted ligation and tested their ability to decode all four valine codons. While all variants translated GUU, GUA, and GUG well, none could efficiently decode GUC. Genome analysis revealed that GUC codons are extremely rare in M. capricolum, with only two coding sequences containing four GUC codons, which the superwobbling tRNA translates efficiently. These findings suggest that the *M. capricolum* $tRNA^{Val}UAC$ is not a "true" superwobbler capable of decoding all four codons equally, but rather a "sufficiently superwobbling" tRNA that has adapted to the organism's codon usage. We are currently extending this study to other minimal-genome organisms, such as *Mycoplasma genitalium* and *Rickettsia prowazekii*, in order to identify "true" and "sufficient" superwobbling tRNAs. This will allow a deeper insight into the phenomenom of superwobbling and a better understanding of decoding in reduced organisms.

Poster 37

Understanding the early stages of Coronavirus infection

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Coronaviruses are enveloped, positive-sense single-stranded RNA viruses. They have been described to cause from mild to lethal infections in mammals and birds, and more recently have been put at the forefront of global attention after the COVID-19 pandemic. Mouse Hepatitis Virus (MHV) is classified within the Betacoronavirus genus, same as SARS-CoV-2, serving as a model to study coronavirus tropism, pathogenicity, and replication.
This project aims to elucidate the very early stages of coronavirus infection, focusing on the events that occur immediately after binding and cellular entry. Understanding how infection is initiated and how replication complexes are established is essential to unravel the molecular basis of coronavirus infection and interaction with the host.
To achieve this, we have implemented a novel live-cell imaging approach based on the SunTag technology, enabling real-time visualization of viral translation events at single-molecule resolution. Compared with conventional fluorescent tagging, this system offers substantially higher sensitivity and temporal resolution, allowing the detection of the earliest stages of infection, including the translation of the first incoming viral genomes.
Ongoing efforts aim to further optimize the system to enhance signal to noise ratio and temporal resolution, enabling comprehensive mapping of the dynamic processes governing the early stages of coronavirus replication. This approach establishes a robust framework for dissecting the molecular mechanisms that drive early coronavirus infection.

Poster 38

Characterization of a *M. tuberculosis* specific GpsI (RNase) inhibitor

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, kills approximately 1.3 million people annually. Current treatment requires a six-month multidrug regimen, but the rise of multidrug-resistant (MDR) strains underscores the urgent need for new drugs with novel mechanisms of action. Ribonucleases have emerged as promising targets, yet high-affinity inhibitors remain scarce.
A whole-cell screen of 400,000 compounds identified nine candidates with anti-mycobacterial activity [1]. Among these, compound X1—1-(4'-(2-phenyl-5-(trifluoromethyl)oxazole-4-carboxamido)-[1,1'-biphenyl]-4-carboxamido)cyclopentane-1-carboxylic acid—showed potent activity in the low micromolar range against intracellular Mtb without host cell toxicity. Whole-genome sequencing of spontaneous X1-resistant mutants revealed single nucleotide polymorphisms in gpsI, an essential gene involved in RNA metabolism.
Our study validates GpsI as the molecular target of X1 and elucidates its mode of action. We demonstrate that X1 specifically inhibits the Mtb RNA decay pathway. Biochemical characterization and Cryo-EM analysis of the ternary enzyme-inhibitor-substrate complex [2] provide structural insights for further optimization.
While transcription inhibitors such as rifampicin target RNA polymerase, their efficacy is increasingly compromised by resistance. The discovery of a selective RNase inhibitor and its essential target represents a significant advance toward a new drug class interfering with RNA degradation. These findings highlight gpsI as a novel drug target and establish X1 as a promising lead compound against Mtb.

[1] Dal Molin M. et. al. J Mol Med (Berl). 2019 Nov;97(11):1601-1613.
[2] Tizian Griesser, Rui Wang, Irene Pachon Angona, Janis Rogenmoser, Julia Obrist, Gisbert Schneider, Peter Sander, Selective inhibition of Mycobacterium tuberculosis GpsI unveils a novel strategy to target the RNA metabolism, Nucleic Acids Research, Volume 53, Issue 11, 24 June 2025, gkaf529

Poster 39

Investigate the role of RNA binding proteins in kidney development and repair using single-cell CRISPR screening and kidney organoids

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Kidney diseases present a growing health burden affecting millions of individuals and leading to rising mortality rates and healthcare costs. Understanding the function of molecular players in kidney development and repair is necessary for establishing novel therapeutic concepts. However, progress has been hampered by a lack of model systems suitable for high throughput characterization of molecular functions.
Using human kidney organoids as a scalable in vitro model, we aim to uncover the gene-regulatory mechanisms that shape kidney development through a pipeline integrating high-throughput genomics, advanced imaging, and genetic screening. Our work will focus on RNA-binding proteins (RBPs), an understudied class of regulators despite their fundamental roles in organ formation and repair, including in the kidney. To achieve a systematic understanding of RBP function, we will pursue three objectives:
(1) Perform single-cell CRISPR screening to identify RBPs that control cell fate decisions during kidney development.
(2) Validate phenotypes combining imaging and genomics .
(3) Map cell type–specific target transcripts of RBPs at single-cell resolution.
In summary, the outlined experiments will (1) establish a novel experimental framework for enhanced molecular discovery in nephrology, and (2) deliver a comprehensive analysis of RBP functions in kidney development and repair, aiming to identify novel molecular targets to prevent or alleviate kidney disease.

Poster 40

Determining the impact of non-coding RNA fragments on innate immune signaling

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Damage-Associated Molecular Patterns (DAMPs) are molecules with particular structural, sequence, and chemical features, which upon release from damaged or dying cells can activate the innate immune system. DAMPs are sensed by Pattern Recognition Receptors (PRRs), which evolved to detect non-self molecules through Pathogen-Associated Molecular Patterns (PAMPs). Constant cell turnover, and especially stress-induced cell damage, causes the release of many molecules, which also includes self-RNAs with DAMP potential on neighboring cells.
This project is testing the hypothesis stating that ncRNA fragments released from damaged cells modulate the innate immune response.
To test this hypothesis, transfection experiments in non-immune cells using poly(I:C), an immunogenic double-stranded RNA, in the absence or presence of ncRNA fragments, were performed. This showed that the immune-stimulating effect of poly(I:C) was suppressed by ncRNA fragments in a dose-dependent manner. Interestingly, ncRNA fragments derived from high-molecular weight RNAs or from low-molecular weight RNAs showed similar suppressive effects in contrast to non-fragmented RNAs. Furthermore, ncRNA fragments grouped according to size, all significantly reduced IFN-β secretion when co-transfected with poly(I:C), which indicates that ncRNA fragment size but not sequence identity determines this effect.
Importantly, this dampening effect was not only sequence- but also RNA modification-independent, suggesting that mass effects of released small RNAs were responsible for these observations.
These results support the hypothesis that cell damage-induced RNA degradation products counteract co-released self-RNAs, thereby contributing to the innate immune system buffering against low-level DAMPs under physiological conditions. Ongoing work aims at determining the molecular mechanisms underlying these effects of ncRNA fragments.

Poster 41

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 69 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.

Poster 42

Exploring biomolecular condensates in bacteria: CsdA forms a nucleoid-associated ribosome biogenesis hub in *E. coli*

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Subcellular organization is essential for maintaining cellular homeostasis. Unlike eukaryotic cells, bacteria lack membrane-bound organelles. Instead, they rely on alternative organizational strategies, including biomolecular condensates. DEAD-box ATPases (DDXs) have emerged as central regulators of RNA-containing condensates in eukaryotes, but whether analogous principles apply in bacteria has remained unknown.
Here we show that *Escherichia coli* employs DDX-driven condensation mechanism to spatially organize ribosomal RNA (rRNA) processing. The cold-shock DEAD-box protein CsdA exhibits a strong intrinsic condensation *propensity in vitro* and forms nucleoid-associated foci in cells at cold temperature. Proteomics and quantitative biochemistry demonstrate that cold-induced upregulation of CsdA protein levels is a key driver of foci formation. Domain dissection further identifies the regions of CsdA that are required for condensation. Affinity purification-mass spectroscopy and super-resolution microscopy reveal that multiple ribosome biogenesis factors interact with CsdA foci at rDNA loci.
In summary these findings suggest that CsdA condensates act as specialized assembly hubs emerging under cold stress to locally enrich factors required for rRNA processing and ribosome maturation. This mechanism resembles the eukaryotic nucleolus and points to an evolutionarily conserved principle by which cells - across the tree of life - use condensation to support ribosome biogenesis.

Poster 43

Experimental identification of preQ1-binding RNAs in the pathogenic bacterium *L. monocytogenes*

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Riboswitches are widespread regulatory RNA modules in bacteria, with many different classes already identified and even more yet to be discovered. Traditionally, the identification of riboswitches has relied on bioinformatic analyses and genetic screens. In our laboratory, we explored the possibility of identifying and characterizing predicted and novel riboswitches using an affinity purification-based approach with a functionalized $preQ_1$ ligand. We successfully enriched a predicted $preQ_1$ riboswitch from *L. monocytogenes* total RNA. Biophysical characterization revealed that this riboswitch can simultaneously bind two ligand molecules and functions as a regulator of translation in vivo. Based on our pull-down approach, we generated a transcriptome-wide pull-down library that showed strong $preQ_1$-dependent enrichment of several candidate sequences. Characterization of one of these candidate mRNAs revealed a $preQ_1$ riboswitch-like sequence in its 5' untranslated region. This potential riboswitch candidate showed a novel way of controlling gene expression upon ligand binding by allowing translation from an alternative start codon located in this region by promoting stop codon readthrough.

Poster 44

A single rRNA-modifying enzyme modulates ribosome fidelity, stability, and tobramycin tolerance in *Vibrio cholerae*

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The massive and inappropriate use of antibiotics has led to their widespread dissemination in the environment, where they are encountered as concentration gradients. While many studies have addressed the effects of lethal antibiotic doses on bacterial physiology, far less is known about how bacteria respond to so-called subinhibitory concentrations (i.e., levels below the minimal inhibitory concentration), even though these are likely the levels most frequently experienced in natural settings. Using *Vibrio cholerae*, a bacterium that alternates between aquatic reservoirs and the human host and therefore faces strong environmental and antibiotic pressures, we found that rRNA modification enzymes play a major role in adaptation to subinhibitory antibiotic exposure [1].
In this study, we focused on one enzyme in particular, RluB, a pseudouridine synthase catalysing the formation of pseudouridine at position 2588 of the 23S rRNA in *V. cholerae*. We showed that deletion of *rluB* leads to increased tolerance to tobramycin, an antibiotic targeting the 30S ribosomal subunit. Analysis of catalytic mutants of RluB further indicated that this phenotype is not driven by the absence of the rRNA modification itself, but rather by the presence of RluB as a protein. Preliminary experiments suggest that ribosomes from the *ΔrluB* mutant may display increased translational fidelity but reduced stability. Ongoing work combines epistasis analyses with other ribosomal components, reporter fusions to quantify translation efficiency, sucrose gradient analyses of ribosome integrity, and omics approaches to define the molecular basis of this phenotype and to clarify how RluB contributes to bacterial adaptation to subinhibitory aminoglycoside exposure.

[1] Babosan A. et al. (2022). Microlife.

Poster 45

Biochemical and Structural Studies of eukaryotic pre-mRNA 3'end processing

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Human protein-coding mRNAs undergo three essential processing steps before they can be exported to the cytoplasm and translated into functional proteins: capping of the free 5′ end, splicing of introns, and cleavage followed by polyadenylation at the 3′ end. The cleavage and polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CStF) complexes are critical components of the multiprotein machinery responsible for 3′-end processing.
This project aims to elucidate the molecular mechanisms of the human 3' end processing complexes. With respect to the CPSF complex, the project aims define the interactions and conformational rearrangements that couple polyadenylation signal recognition with pre-mRNA cleavage. A parallel research aim focuses on the functional relevance of dimerization within the cleavage stimulation factor (CStF) complex. The project relies on in vitro biochemical reconstitution of the complexes and structural analysis by cryo-EM.

Poster 46

Transcriptome-wide detection of differential RNA editing using LoDEI

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A-to-I editing is a major modification of RNA that is thought to affect the stability of RNA structures, the localization and splicing of mRNA, the targeting of microRNA, and protein synthesis. This process is mediated by double-stranded RNA-specific adenosine deaminase (ADAR), which is often overexpressed and associated with tumorigenesis and innate immune response. Long dsRNA elements often found in the 3'-UTR of mRNAs are bound and edited. Academic and industrial researchers are interested in the functional implications of A-to-I editing, as the fast-growing number of clinical studies applying base editors underscores. However, analyzing the dynamics of A-to-I editing in a biological or therapeutic context requires sensitive detection of differential A-to-I editing, which is currently an unmet need.
We present the local differential editing index (LoDEI, PMID: 39443485), a novel approach to detecting differential RNA editing in RNA-seq datasets. Our method utilizes a sliding-window strategy in conjunction with an empirical q value calculation, a technique that identifies a greater number of RNA editing sites at the same false-discovery rate compared to existing methods. As revealed by LoDEI, we demonstrate for the first time that the oncogene MYCN increases, while a specific small non-coding RNA reduces A-to-I editing.

Torkler P. et al (2024) Nat Commun 15, 9121

Poster 47

Deciphering the role of SUMOylation during the programmed genome rearrangement in *Paramecium tetraurelia*

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SUMOylation is a post-translational protein modification through attachment of a Small Ubiquitin-like MOdifier at lysine residues. It is implicated in regulation of protein trafficking and stability. However, its function in ciliates remains unclear. Previous research hinted at roles of SUMOylation in the programmed genome rearrangements during macronuclear development in Paramecium tetraurelia. Here, we show the presence of SUMOylation using Sumo3 in the newly forming somatic nuclei during sexual reproduction of Paramecium. Furthermore, we found SUMOylation as essential for DNA excision during developmental genome rearrangements and silencing of Sumo proteins results in the retention of internal eliminated sequences. Our goal aims to uncover the roles of SUMOylation in ciliates and understand how it regulates genome excisions. To achieve this, we obtain a complete SUMOylome during the early developmental stages of sexual reproduction. This comprehensive approach has identified SUMOylated proteins that play a critical role in genome rearrangement, providing a foundation for elucidating the intricacies of this mechanism.

Poster 48

Impact of A-to-I editing on m^{6}A deposition

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N^6-methyladenosine (m^{6}A) and adenosine-to-inosine (A-to-I) RNA modifications play critical roles in post-transcriptional gene expression regulation and thereby control important cellular processes. Moreover, both modifications can modulate the recognition of RNAs by pattern recognition receptors. However, our understanding of the crosstalk between these two RNA modifications is currently limited. On a global scale, the impact of m^{6}A modification on A-to-I distribution has been demonstrated before. In contrast, the influence of A-to-I editing on m^{6}A modification remains unclear. In this study, we use wild-type and ADAR-deficient mouse brain tissue to determine changes in m^{6}A deposition using two orthogonal methods, MeRIP-Seq and MAZTER-seq, in the presence and absence of A-to-I editing. By combining these two techniques, we demonstrate reproducible changes in m^{6}A levels in a subset of transcripts in the mouse brain. Selective introduction of inosines in one of the identified, ectopically expressed substrates using site-directed RNA editing, we can prove that A-to-I editing can affect m^{6}A deposition in cis. Thus, we can demonstrate that the deposition of inosines can directly affect m^{6}A levels at nearby sites.

Poster 49

ZCCHC9 is a human SSU processome factor required for ENP1 loading and nucleolar 18S rRNA maturation

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In human cells, the highly choreographed steps of ribosome biogenesis take place in the nucleolus, the nucleoplasm and the cytoplasm, and are facilitated by hundreds of transiently acting ribosome biogenesis factors (RBF). This intricate maturation process is initiated with the transcription of a long polycistronic precursor ribosomal RNA (pre-rRNA). Upon the co-transcriptional association of ribosomal proteins (RP) and early-joining RBFs, a large pre-ribosomal particle emerges, known as the small subunit (SSU) processome. Here, we performed affinity purification-coupled mass spectrometry (AP-MS) from nucleolar extracts to shed light on the molecular composition of the SSU processome and identified the previously uncharacterised protein ZCCHC9 as a novel SSU processome-associated RBF. Follow-up analyses by immunofluorescence, polysome profiling and Northern blotting revealed that the depletion of ZCCHC9 results in an early nucleolar 40S biogenesis defect. By exploiting AP-MS analysis and Alphafold3-based interface predictions, we identified EMG1 as a candidate docking partner of ZCCHC9 on the SSU processome, which is supported by proximity labelling experiments, as well as biochemical and functional analyses. Finally, we demonstrate that the EMG1-dependent recruitment of ZCCHC9 is essential for the incorporation of ENP1 into the SSU processome, explaining the role of ZCCHC9 in nucleolar 40S subunit maturation.

Poster 50

Tumour mutations remodel oncogenic non-coding RNA:protein networks

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Hundreds of long noncoding RNAs (lncRNAs) promote cancer through dysregulated expression, yet whether these loci are targeted by fitness-altering somatic driver mutations remains unclear. Discovery of such 'driver lncRNAs' has been constrained by the limited size of tumour genome cohorts. I will describe a map of 121 mutated driver lncRNAs based on the largest available collection of 13,280 whole-genome sequences from Genomics England. Their oncogenic roles are supported by diverse evidence including functional screens and mutual exclusivity with established protein-coding drivers. Mechanistically, we have found that driver mutations target functional elements including RNA-binding protein (RBP) interaction sites within established onco-lncRNAs including MALAT1 and NEAT1. These mutations alter RBP recruitment to enhance onco-lncRNA activity through a variety of means including dysregulated epitranscriptomic modifications and promotion of membraneless organelles. Our results reveal critical roles of ncRNAs at the most fundamental steps of cancer progression.

Poster 51

How do TERRA R-loops promote homology-directed repair at telomeres?

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Telomeres are essential for maintaining genome stability. Due to the end replication problem, they shorten with every round of DNA replication. In cancer cells, preserving telomeres is essential for enabling their immortality. Most cancer cells maintain their telomeres through telomerase. However, in approximately 10-15% of cancers, cells employ a telomerase-independent mechanism, specifically a recombination-based pathway called Alternative Lengthening of Telomeres (ALT).
TERRA, a long non-coding RNA transcribed from telomeres, can invade telomeres and form RNA-DNA hybrid structures known as R-loops. TERRA R-loops are known to be essential for telomere maintenance via HDR (Homology-Directed Repair) in ALT cancers. However, the precise connection between TERRA R-loops and HDR remains unclear. In particular, it is undetermined if TERRA R-loops elicit telomere damage which is repaired by HDR or if TERRA R-loops also directly stimulate the HDR reaction.
We use POT1-knockout cells to investigate the relationship between TERRA R-loops and HDR. POT1 loss leads to HDR-mediated telomere elongation, increased TERRA R-loops, and the recruitment of several RNA-binding proteins to telomeres [1]. Here, we focus on SYNCRIP, an RNA-binding protein that is enriched at telomeres after POT1 loss. Depleting SYNCRIP in POT1-deleted cells suppresses HDR-mediated telomere elongation and appears to reduce R-loop levels. By manipulating TERRA R-loops via SYNCRIP, we should soon be able to identify the exact functions of TERRA R-loops for HDR-mediated telomere maintenance.

[1] Glousker G. et. al. EMBO J. 2020;39(23):e104500.

Poster 52

DarT2 ortholog mining yields flexible Append Editors

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The targeting of single point mutations to treat genetic diseases has an immense therapeutic demand. Our lab has developed a novel base editor, termed Append Editor (AE), that mediates T-to-A and T-to-C base conversion via targeted DNA ADP-ribosylation in eukaryotic cells, both mechanism and editing outcome being so far unique in base editing. However, the technology is limited by the sequence dependency of the employed DNA ADP-ribosyltransferase DarT2 which only targets thymines within in TYT motif context. Here, we use ortholog mining combined with cell-free high-throughput NGS screening to identify DarT variants with relaxed motif recognition. We then onboard interesting candidates for Append Editing in mammalian cells and demonstrate flexible, previously-unachievable thymine base conversion. Our optimization paves the way for translating AE into a versatile therapeutic tool to treat detrimental genomic disease, for instance Sickle Cell Disease (SCD).

[1] Gupta D. et. al. (2025) Nat Biotechnol. doi: 10.1038/s41587-025-02802-w

Poster 53

Imaging HIV-1 transcription in live latent T cells reveals rare viral transcriptional bursts

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HIV-1 transcription is stochastic with bursts of transcription occurring sporadically in absence of Tat. Recent studies clearly demonstrated that in patient cells, transcriptional reactivation of latent viruses by external signals is stochastic as well, with higher rate of reactivation occurring after several rounds of stimulation. While efforts have been made to characterize the stochastic fluctuations of HIV-1 transcription and their determinants, the transcriptional activity of latent proviruses in T cells remains uncharacterized. We hypothesize that latent proviruses may exhibit sporadic pulses of viral transcription that could contribute to latency dynamics and viral rebounds. These pulses may generate sufficient Tat to induce full viral activation leading to latency exit, or may produce a limited number of viral particles capable of infecting new cells despite incomplete viral activation. To investigate this process, we developed an MS2-tagged dual-color HIV-1 vector that enables both the selection of latently infected cells and live-cell imaging of viral transcription with single-molecule sensitivity. Using this system, we imaged viral transcription in a latent T cell clones carrying an intact viral genome under basal conditions and upon activation. Under basal conditions, latent cells exhibited rare bursts of transcriptional activity. Each burst produced only a few RNAs and was separated by inactive periods lasting several hours, underscoring the extremely low transcriptional activity of latent viruses. Latent cells also displayed substantial cell-to-cell transcriptional heterogeneity. Importantly, we showed that basal viral transcription is insufficient to trigger spontaneous latency exit.

Poster 54

SARS-CoV-2 Nucleocapsid relies on Phase Separation to selectively form viral RNPs inside Condensates

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SARS-CoV-2 nucleocapsid (N) protein is crucial for genome packaging and one of the minimal components of the viral ribonucleoprotein (vRNP) particle. Here, we systematically investigated the specificity and the physicochemical driving forces of liquid-liquid phase separation (LLPS) of N protein and the role of LLPS for vRNP formation. We show that condensate formation of N protein with nucleic acids is driven by an intricate balance of electrostatic and hydrophobic interactions, which largely depends on the chemical environment and thus allows finetuned regulation of the process during the viral life cycle. The important regulatory role of LLPS is further corroborated by the ability of N:RNA condensates to undergo context-dependent noise buffering. Despite non-specific LLPS, N protein selectively forms vRNP complexes with GU-rich viral RNAs. In fact, LLPS allows vRNP formation and compaction, and condensates were found to protect RNA from degradation. Combined, our results enhanced our mechanistic understanding of vRNP formation and highlight the role of LLPS during genome packaging – both of which have important implications for the potential druggability of SARS-CoV-2 infections.

Poster 55

High-resolution mapping of rRNA modifications in *Bacillus subtilis*

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Ribosomal RNA (rRNA) undergoes various post-transcriptional chemical modifications (PTMs) during ribosome biogenesis, with pseudouridine (Ψ) and 2′-O-methylation of ribose being among the most common. These chemical marks contribute to ribosome stability and translation fidelity. Recent high-resolution structures obtained by X-ray crystallography and cryo-electron microscopy (cryo-EM) have enabled direct visualization of many ribosomal PTMs across diverse model organisms, including *E. coli* (PDB 8B0X, 1.55 Å), *T. thermophilus* (PDB 8FC1-6, ~2.5 Å), *L. donovani* (PDB 6AZ1/3, 2.5 Å), *S. aureus* (PDB 8P2G, 2.02 Å), and human (PDB 8QYX, 1.78 Å).
In this study, we combined cryo-EM, Next-Generation Sequencing and Nanopore sequencing to characterize and map rRNA PTMs in *Bacillus subtilis*, a Gram-positive model bacterium. We identified 25 modifications across the 16S and 23S rRNAs, along with the enzymes responsible for their installation. Most PTMs were directly observed in our 1.7 Å cryo-EM map and independently confirmed by sequencing data. Targeted gene deletions, combined with analysis of modification profiles, enabled us to pinpoint the enzymes responsible for each modification.
This work provides the most comprehensive map to date of the *B. subtilis* rRNA modification landscape. These findings establish a definitive reference of *B. subtilis* rRNA PTMs and open new avenues for dissecting how RNA modifications fine-tune bacterial translation. Beyond bacterial physiology, this resource also offers a valuable framework for exploring how conserved RNA-modification pathways may influence host–microbe interactions and contribute to human disease.

Poster 56

Computational approaches for studying readthrough transcripts biogenesis and functions in neuroblastoma cells

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Correct transcription termination, which relies on the recognition of the transcription termination site (TTS), is an essential step in gene expression. Under cellular stress or in diseases, the TTS is occasionally not recognized, allowing transcription to continue beyond its normal boundary (readthrough transcription). When two genes are genomically positioned in tandem, readthrough transcription can invade the downstream gene, generating transcription readthrough-associated chimeric RNAs (tracRNAs). The DEAD box helicases DDX5 and DDX17 are key regulators of RNA metabolism, transcription termination [1] and RNA/DNA structure modulation. In neuroblastoma, where the MYCN oncogene is frequently amplified, the team showed that DDX17 and MYCN interact and that DDX5 and DDX17 depletion increases tracRNA production [2].
This project aims to elucidate the mechanisms linking helicases activity (especially on G-quadruplex) and readthrough transcription and to characterize the role of tracRNAs in neuroblastoma. Our mechanistic approach will integrate mutli-omics data, focusing on long read sequencing and G4 mapping to understand TTS recognition regulation. The functional part of the project will involve studying the non-coding functions of tracRNAs (as miRNA sponges or interacting with RNA binding proteins) and characterizing coding tracRNAs to assess their potential neoantigen production. Finally, we will develop a comprehensive computational atlas of tracRNAs which will be built from cell line data and enriched with patient tumor data. The goal is to better characterize neuroblastoma-specific tracRNAs and provide a resource for identifying tracRNAs common to other cancer types.

[1] S. Terrone et al., 2022, Nucleic Acids Res
[2] V. Clerc et al., 2025, Research Square

Poster 57

Polyamines and codon-specific translation regulation in cancer

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Cancer initiation and progression is commonly accompanied by global reprogramming of the proteome. Only a fraction of those changes are explained by mRNA abundance, pinpointing to additional layers of regulation.
Here we identify polyamines as modulators of oncogenic translation. In a high-risk neuroblastoma mouse model, DFMO-mediated polyamine reprogrammed mRNA translation to induce cellular differentiation and significantly improved survival [1]. This translational reprogramming was marked by ribosomal slowdown at adenosine (A)-ending codons which are enriched in cell-cycle genes and depleted in differentiation-associated transcripts.
Translation rate and fidelity are modulated by tRNA modifications and the abundance of charged tRNAs matching a given codon pool [2]. As decoding of A-ending codons requires wobble–modified tRNAs, we assessed whether altered tRNA modification contributed to the observed effects. Nucleoside mass spectrometry showed no global changes, except for queuosine-related modifications. Using mim-tRNAseq to profile tRNA abundance and charging revealed differential expression of some isodecoders after polyamine depletion, but no net change in overall decoding capacity. Furthermore, DFMO treatment increased deacetylated tRNAs, yet aminoacylation levels did not correlate with codon-specific slowdown.
Thus, polyamine depletion alters decoding kinetics between wobble base modified tRNAs and their cognate codons independent of global tRNA modification, abundance, or charging. We further plan to quantitatively profile epigenetic marks on tRNA, mRNA and rRNA in combination with stable-isotope tracing in vitro and in vivo. This will reveal dynamic changes in turnover, modification status, and metabolite source, fostering our understanding of the intricate interplay between metabolism, RNA biology, and translation in cancer.

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Poster 58

SRSF1 condensates enhance splicing by two mechanisms: RNA protection and surface exposure

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Biomolecular condensates have emerged as central regulators of numerous molecular functions. Through a mechanism known as liquid-liquid phase separation, proteins containing low-complexity domains, such as splicing factors, are prone to formation of condensates. Although these condensates are biophysically well characterized, their implication in pre-mRNA splicing remains unclear [1-3]. Here, we uncover a novel mechanism explaining how condensates enhance splicing. We show that serine-arginine-rich splicing factor 1 (SRSF1) and hnRNPA1 coexist in condensates, and addition of RNA results in their spatial segregation. Phosphorylation of SRSF1 RS domain, which is critical for its splicing [4], induces a reorganization of the condensates and a relocalization of RNA with hnRNPA1. Addition of a mimic of U1snRNP pulls the 5' splice site (ss) to the interface between hnRNPA1 and SRSF1 droplet, showing that condensates of splicing regulators modulate spatial organization of ss. Functional assays reveal that the phosphorylation of SRSF1 regulates the localization of the pre-mRNA within condensates and ultimately modulates the splicing outcome. Under low phosphorylation state, SRSF1 can protect RNA by sequestering it within the core, resulting in low splicing efficiency. In contrast, under higher phosphorylation, both SRSF1 and pre-mRNA relocate to the condensate surface, leading to higher splicing efficiency. We propose that SRSF1 protects RNA by sequestering it within the condensed phase and upon SRSF1 phosphorylation, RNA relocalizes to the droplet periphery, promoting its interaction with the splicing machinery. Historically viewed as passive storage sites, splicing factor containing condensates portray here a novel and active role in splicing regulation.

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Poster 59

Extended Shine-Dalgarno motifs govern translation initiation in *Staphylococcus aureus*

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Regulation of translation initiation is central to bacterial adaptation, but species-specific mechanisms remain poorly understood. We present high-resolution mapping of translation start sites in *Staphylococcus aureus*, revealing distinct features of initiation alongside numerous unannotated small ORFs. Our analysis, combined with cryo-EM of a native mRNA-ribosome complex, shows that *S. aureus* relies on extended, start codon proximal Shine-Dalgarno (SD) interactions, creating specificity against phylogenetically distant bacteria. Several natural *S. aureus* initiation sites are not correctly decoded by *E. coli* ribosomes. We identify new and conserved non-canonical start codons, whose regulatory initiation sites contain these characteristic extended SD sequence motifs. Finally, we characterize a novel example of uORF-mediated translational control in *S. aureus*, demonstrating that translation of a small leader peptide modulates expression of a key biofilm regulator. The described mechanism involves codon rarity, ribosome pausing and arginine availability, linking nutrient sensing to biofilm formation in this major human pathogen.

Poster 60

On the chemical mechanism of the twister-sister ribozyme

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The mechanism by which the twister-sister ribozyme catalyzes site-specific cleavage of its own phosphodiester backbone remains unresolved [1,2]. Although crystallographic studies [3,4] have provided insights into the structure, folding, and function of this four-way junctional RNA, the key determinants underlying its exceptional rate enhancement are still unknown. Our work aims to identify these determinants using atomic mutagenesis: by individually substituting functional groups at conserved nucleosides in the active site [2] and comparing the resulting cleavage activities, we quantify the catalytic contribution of each group. This approach leverages a toolbox of deaza-nucleobase–modified phosphoramidites that we have synthesized in recent years, enabling solid-phase synthesis of RNAs with the required mutations. In this study, we particularly focus on modifying an active-site guanosine to test interactions inferred from crystal structures, specifically hydrogen bonding with the scissile phosphate. We are also extending our analysis to assess the roles of divalent metal ions at backbone-binding sites near the scissile phosphate observed in the pre-catalytic state [3]. To this end, we present preliminary results on the effects of individual thiophosphate and methylphosphonate substitutions. Together, these findings advance our understanding of the catalytic mechanism of four-way junctional twister-sister ribozymes.

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Poster 61

Characterization of Translating and RTC-associated Nsp3 in Early CoV Infection

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Early events in the Coronavirus (CoV) replication cycle following spike-mediated entry and RNA release are poorly understood. Little is known about primary translation of the incoming RNA genome, the first round of replication and transcription, and the secondary translation of progeny genomes and subgenomic RNAs. Central to these early steps is the viral replicase-transcriptase complex (RTC), which forms through primary translation and establishes RNA synthesis within double-membrane vesicles (DMVs). Nsp3 is a key RTC component that reorganizes ER membranes to generate DMVs. It localizes to their outer membrane, and participates in a hexameric pore enabling RNA transport.
Because most existing data derive from later infection stages, we aim to characterize nsp3 translation, its role in RTC assembly, and DMV formation during early coronavirus infection using single-molecule imaging and electron microscopy. We established the SunTag protein-tagging system for CoVs, fusing a peptide array to nsp3 to visualize single nsp3 translation events via GFP recruitment. We detect the first translation events within 60 minutes of infection and observe fluorescent foci that grow in size and intensity over time. We propose that small foci represent active translation, while larger foci reflect accumulated nsp3 within the RTC. By using translation inhibitors or by removing the Suntag after primary translation, we will distinguish translation-derived foci from RTC-associated ones.
In parallel, we fused APEX peroxidase to nsp3 to enable EM-based detection of its spatial association with DMVs. Together, these approaches will refine our understanding of early RTC formation, nsp3 dynamics, and potential CoV recombination during co-infection.

Poster 62

Codon usage and tRNA modifications in the regulation of quiescence

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In response to environmental changes or stress, cells can exit the cell cycle and enter a reversible, non-dividing state known as quiescence. During this state, cells substantially change their gene expression, metabolism, and biophysical properties, allowing them to retain the capacity to re-enter the cell cycle even after extended periods of time [1]. In yeast, nutrient limitation like glucose starvation is a well-established trigger for quiescence. A hallmark of this transition is a transcriptional and translational shift from pro-proliferative to stress-responsive genes. To rapidly produce stress-induced proteins, quiescent cells prioritize translation of newly transcribed mRNAs [2]. Intriguingly, stress-related genes that are translationally upregulated during starvation display a different codon usage than downregulated genes, suggesting a mechanism by which cells distinguish stress mRNAs from proliferative mRNAs. Furthermore, the loss of U34 wobble uridine tRNA modifications reduces the regrowth capacity of yeast cells upon entry into quiescence. We hypothesize that both tRNA usage and modifications contribute to the efficient translational switch during quiescence. To test this, we investigate whether codon optimality shifts during glucose starvation to favour the translation of stress-related transcripts. Using reporter constructs and translation elongation speed assays in wild type and U34 wobble uridine-deficient mutant strains, we aim to elucidate the role of tRNA modifications in the translational regulation of quiescent cells.

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Poster 63

Liquid chromatography – mass spectrometry analysis of RNA modifications

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Chemical modifications of the base or sugar moiety extend the functional diversity of canonical RNA nucleotides. These alterations can affect all aspects of RNA metabolism, including stability, splicing, and translation efficiency. The resulting changes can in turn influence gene expression, stress responses, and lead to diseases. In recent years, several human pathologies, including cancer and metabolic disorders, have been linked to the dysregulation of RNA modification landscapes. As interest in the epitranscriptome grows, precise, quantitative methods for profiling RNA modifications have become crucial. While sequencing methods can only map a small subset of modifications, Liquid Chromatography-Mass Spectrometry (LC-MS) remains the gold standard for accurate global quantification due to its specificity, sensitivity, and ability to distinguish closely related nucleosides.
We have developed a powerful analytical platform for the comprehensive quantitative profiling of RNA modifications. Following enzymatic digestion and high-pressure chromatographic separation, the modified nucleosides are identified by their specific mass-to-charge and fragmentation patterns. Our Thermo Scientific Exploris 480 mass spectrometer provides unparalleled sensitivity and allows for the simultaneous analysis of multiple modifications from limited sample quantities. In addition to monitoring a wide range of known modifications, we offer targeted assessments of predicted modifications.

Poster 64

Programmable Gene Silencing in *Enterococcus faecalis*: From Genetic Toolkit to Next-Generation ASObiotics

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Enterococcus faecalis remains a challenging organism for genetic manipulation, creating a bottleneck in our understanding of its pathogenicity and antibiotic resistance mechanisms, particularly in Vancomycin-Resistant Enterococci (VRE). To overcome this barrier, we have developed a transcript-targeting platform using Peptide Nucleic Acid (PNA) antisense oligomers (ASOs). This approach bypasses the need for complex genetic engineering, providing a rapid method for phenotypic analysis.
We report the successful delivery and target engagement of PNA-ASOs in *E. faecalis*. Building on this validation, we are deploying the platform for two critical applications. First, we target essential metabolic pathways to induce growth arrest, validating the PNA-ASOs as a novel class of antimicrobial agent. Second, we utilize the sequence specificity of ASOs to selectively inhibit resistance genes, effectively breaking VRE resistance and resensitizing the pathogen to vancomycin. This work expands the genetic toolkit available for *E. faecalis* and demonstrates the translational potential of antisense technology in reshaping the treatment landscape for multidrug-resistant infections.

Poster 65

Investigating dynamics of tRNA abundance and modification during embryo development

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Until recently, relatively little was known about the expression of critical adaptor molecules, transfer RNAs (tRNAs) during embryo development. However, recent advances in NGS technologies have enabled the mapping of tRNA expression and modifications profiles, which allowed our lab to show that these profiles are dynamically altered during gastrulation in zebrafish [1]. This observation points towards an adaption in the translation machinery during the different phases of an early embryo development.
In this project, we utilize a model based on mouse embryonic stem cells to recapitulate early mammalian embryo development in vitro. We constructed NGS libraries, assaying tRNA expression and modification profiles throughout the differentiation process. We observed that throughout differentiation, tRNA expression and modification content dynamically undergoes alterations.
We now aim to investigate the contribution of the genomic context on the differentially expressed tRNA genes. Furthermore, we will investigate the impact of spatial or temporal perturbation of the tRNA repertoire in embryo development.
We ultimately aim to shed light on the physiological relevance of the diverse tRNA transcriptome during differentiation.

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Poster 66

Unraveling the mechanisms by which plant and synthetic small non-coding RNAs direct gene silencing in bacteria

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Small RNAs (sRNAs) can be transported between interacting organisms and, in some instances, trigger gene silencing in recipient cells. This cross-kingdom RNA interference process has been well studied in plant-fungal interactions and occurs in a bidirectional manner. Intriguingly, we have demonstrated that Arabidopsis transgenic plants expressing sRNAs directed against a key virulence factor of *Pseudomonas syringae* pv. tomato strain DC3000 (*Pto* DC3000) direct gene silencing of this target and the dampening of pathogenesis. Three populations of apoplastic extracellular sRNAs were found biologically active. The first one is non-vesicular and associated with proteins, whereas the second one is located inside extracellular vesicles. The third population is unbound to proteins and in a dsRNA form. However, the mode of action of these antibacterial sRNAs over their cognate targets, and the machinery responsible for gene silencing in *Pto* DC3000 remain unknown. To address this, we established a semi-in vivo assay using chemically synthesized sRNAs targeting a *Pto* DC3000 virulence factor. Using this approach, we found that 21nt long sRNAs, in a dsRNA form and composed of 2-nt 3' overhangs at both ends, and single-stranded sRNAs exhibiting sequence complementarity with its target, were found effective. I will present the results obtained with these different antibacterial sRNA species and report on approaches currently developed to identify the machinery responsible for sRNA-directed gene silencing in *Pto* DC3000 as well as the rules governing the principle of sRNA target recognition.

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Poster 67

Understanding the physiological and pathological roles of the tRNA deaminase complex ADAT2/ADAT3 during cortical development

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Transfer RNAs (tRNAs), adaptor molecules involved in translation, are the most modified RNA species. These modifications, catalyzed by numerous tRNA modifying enzymes, influence tRNA structure, function and stability depending on their chemical nature and position on the tRNA. Interestingly, mutations in genes coding for tRNA-modifying enzymes have been increasingly associated to neurodevelopmental diseases (NDDs). Yet, the biological role of these modifications and the impact of mutations identified in patients to the pathophysiology of NDDs remains poorly characterized.

The ADAT2/ADAT3 complex catalyzes the adenosine to inosine modification at the wobble position 34 of tRNAs starting with an A in their anticodon (ANN-tRNAs). Although mutations in *ADAT3*, the catalytically inactive member of the ADAT2/ADAT3 complex, have been identified in patients with severe neurodevelopmental disorders (NDDs), the impact of those mutations on the complex and during brain development remain poorly characterized. Using patient-derived cells and performing an Adat2/3 knockdown specifically in mouse embryonic neurons, we previously showed that 1) I34 levels as well as the steady state levels of ANN-tRNAs are decreased in patient's cells and 2) catalytic activity of the ADAT complex is required for proper migration of projection neurons during cortical development.

Here, we generated a conditional double knock-out (KO) mouse model in which both *Adat2* and *Adat3* are no longer expressed in cortical progenitors and in the neurons they produce. Preliminary results show that absence of the complex during cortical development leads to a smaller brain. This likely results from increased cell death and accumulation of aggregated proteins.

Poster 68

Regulatory mechanisms of mRNA sequestration and fate in P-bodies

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Cells rapidly adjust to environmental changes by modulating their gene expression at both the transcriptional and translational levels. Processing Bodies (PBs) are dynamic, stress-induced membrane-less organelles containing mRNA and RNA decay factors. They are thought to regulate cytoplasmic mRNA fate by serving as sites of storage or decay. We have previously established that the mRNA content of PBs is stress-dependent. To investigate the context-specificity and temporal changes of PBs, we expanded the stress repertoire beyond the canonical glucose starvation to include ER and mitochondrial stressors, as well as redox stresses under both acute and prolonged exposure. We applied chemical cross-linking coupled to affinity purification (cCLAP), followed by proteomics and RNA sequencing, to capture stress-specific PB-associated proteins and transcripts *in vivo*. These datasets provide an unprecedented wealth of information on mRNA metabolism under a variety of stresses both acute and after adaptation. A subset of mRNAs for each stress condition will be verified by MS2 live-cell imaging. We apply sequence and structure motif analyses to identify regulatory elements governing selective mRNA sequestration. Combining the *in vivo* and *in silico* approaches, we aim to elucidate the regulatory mechanisms of mRNA recruitment to PBs. By linking PB composition to individual stress response, we will be able to understand the grammar that dictates the fate of an mRNA between translation, storage, and decay.

Poster 69

Deciphering the functional interactions between RNA-binding proteins and the miRISC complex

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MicroRNAs (miRNAs) are short non-coding RNAs of 20–24 nucleotides that bind to Argonaute proteins forming the miRNA-induced silencing complex (miRISC) and mediate repression of target messenger RNAs (mRNAs). The most efficient silencing typically occurs at binding sites complementary to nucleotides 2–8 of the miRNA and located within 3′ UTRs. These regions also contain binding sites of numerous RBP; many sites are immediately adjacent to predicted miRNA binding sites or even overlap. Such spatial proximity suggests that RBPs may influence miRISC recruitment: either synergistically or antagonistically. Despite growing evidence of functional interplay between RBPs and miRISC, the molecular rules governing these interactions remain poorly understood. To address this gap, we use single-molecule in vitro approaches to determine the binding hierarchy and kinetic parameters, and ex-vivo cellular systems to define regulatory consequences of RBP-miRISC interactions. To estimate the extent of potential RBP–miRISC interactions across the transcriptome and rationally choose candidates for our studies, we mapped all mouse miRNA 8-mer seed sequences and RBP consensus binding motifs across approximately 80,000 annotated 3′ UTRs. For each potential triplet (RBP–miRNA–target mRNA), we retained overlapping and proximal binding sites; for proximal sites, we predicted RNA secondary structures to assess spatial proximity. We then filtered candidate triplets using cell-type-specific expression atlases to ensure physiological co-expression. Based on these predictions, we are now producing recombinant RBPs, including TRIM71, FMR1, PUM1/PUM2, and HuR, alongside AGO2 miRISCs. Our findings may reveal mechanistic insights into the functional interactions between RBPs and miRISCs and their roles in combinatorial post-transcriptional regulation in cells.

Poster 70

RNA-PROTACs in ALS: Toward Selective Degradation of TDP-43

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative disorders marked by cytoplasmic protein aggregation. TDP-43, an RNA-binding protein, forms toxic aggregates in ~97% of ALS and ~50% of FTD cases [1], making their selective removal a promising therapeutic strategy. RNA-PROTACs (oligonucleotide-based proteolysis-targeting chimeras) enable selective degradation of RNA-binding proteins and have shown efficacy against FUS aggregates [2], another ALS-related protein. However, their application to TDP-43 remains limited [3]. Here, we evaluated a candidate RNA-PROTAC (AUG12-PEG2-Pomalidomide) in human neuron-like SH-SY5Y cells, focusing on phosphorothioate stereochemistry to optimize binding to TDP-43 RNA-binding domains. Specifically, we assessed efficacy, putative toxicity, internalization dynamics, subcellular localization, and potential TDP-43 loss-of-function across multiple concentrations and time points. AUG12-PEG2-Pomalidomide proved to be effective in reducing the total amount of TDP-43 up to 70% following transfection. Nearly all treated cells (98.9%) internalized AUG12 strands via gymnosis at 125 nM, with signal progressively decreasing over time, indicating oligo degradation. Both AUG12 and AUG12-PEG2-Pomalidomide exhibited similar uptake and predominantly cytoplasmic distribution, co-localizing with lysosomes. In contrast, oligo-only controls showed stronger nuclear signals. Importantly, none of the tested RNA-PROTACs triggered TDP-43-mediated loss-of-function under physiological conditions. Moreover, preliminary experiments in iNets, a multicellular system comprising neurons, astrocytes, and oligodendrocytes [4], suggested a predominantly nuclear localization of AUG12 in normal neurons. Next, we will test these RNA-PROTACs in Ngn2-derived motor neurons with TDP-43 pathology to evaluate their localization and activity in a disease-relevant context. Altogether, these findings lay the groundwork for RNA-PROTAC-based strategies targeting TDP-43 in ALS and FTD.

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Poster 71

RNA binding and condensate formation by translation initiation factor eIF4B

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The eukaryotic translation initiation factor eIF4B is involved in the efficient translation of mRNA with structured 5' regions, such as a subset of mRNA implicated in cancers, and also plays a role in stress granule regulation. Due to the large proportion of intrinsic disorder in eIF4B, few structural details have been determined for eIF4B except for its single folded RRM domain. Using a suite of biophysical approaches, including NMR spectroscopy, we have recently characterized the ability of the C-terminal intrinsically disordered region of eIF4B to mediate separate roles in oligomerization and condensate formation, and mapped its complex self-association landscape. We have now focused on the RNA-binding properties of eIF4B by the intrinsically disordered regions and the folded RRM domain. Using NMR spectroscopy, single-molecule FRET and other approaches we have characterized a surprisingly strong binding preference for guanosine-rich sequences for several regions of intrinsically disordered residues, and an affinity that scales with condensate formation. The G-rich specificity also continues for the RRM domain and together with the IDR interactions provide molecule details of RNA effects on the eIF4B protein behaviour at the nanoscale and mesoscale levels.

Poster 72

Mass spectrometry analyses of pre-ribosomal 40S intermediates reveal a recruitment platform for processing enzymes

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Eukaryotic ribosome biogenesis begins in the nucleolus, where ribosomal (r)RNA is transcribed and assembled into ribosomal precursors. Their continued maturation is mediated by numerous factors and coupled to passage from nucleolus through the nucleoplasm to the cytoplasm. Here, we investigated assembly of human small ribosomal subunits by engineering endogenously tagged cell lines for several factors involved at different maturation stages. Their affinity purification from nucleolar, nuclear and cytoplasmic extracts followed by mass spectrometry allowed us to obtain interactomes across compartments. Combined with published structures and AlphaFold predictions, these datasets provide an approximate spatio-temporal atlas of human 40S ribosome assembly and suggest new direct interaction partners.
In follow-up analyses, we show that RRP12 serves as a docking platform for two rRNA maturation enzymes, exonuclease PARN and RNA methyltransferase RRP8. We define the molecular basis of their direct interactions and demonstrate that RRP8 is not only involved in large but also small subunit biogenesis. Furthermore, we reveal that association of PARN with RRP12 is required for its recruitment to nucleoli and successful trimming of rRNA precursor 18S-E. Thus, RRP12 functions as a substrate adaptor for PARN by tethering it to the correct rRNA precursor on which to perform its exonuclease function.

Poster 73

SINV Infection and Oxidative Stress Trigger Dicer Redistribution to Cytoplasmic Foci in Myotis myotis cells

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Bats are unique mammals with remarkable physiological and immunological characteristics. As the only mammals capable of powered flight, they endure substantial stress and metabolic constraints. They are also known for their ability to harbor various pathogenic viruses without developing clinical symptoms. These distinctive traits raise important questions about the molecular mechanisms underlying bat immunity and stress resistance.
Dicer, best known for its role in miRNA biogenesis, also participates in DNA repair, stress regulation, and RNAi in plants and invertebrates. The exact contribution of antiviral RNAi in mammals remains to be determined, but Dicer has been shown to modulate innate immunity in a non-canonical manner. Interestingly, recent studies have highlighted an increased RNAi-dependent antiviral role of Dicer in two bat species' cell lines. However, the canonical and non-canonical roles of Dicer in other bat species remain largely unexplored.
Using a nasal epithelial cell line from *Myotis myotis* (Mm-NE), we investigated the behavior of Dicer during viral infection and cellular stress. We found that Dicer relocalizes to cytoplasmic foci during Sindbis virus (SINV) infection and oxidative stress. Our results suggest that Dicer translocates to viral factories during late stages of SINV infection and to stress granules at early stages or under oxidative stress.
Notably, Dicer appears to exhibit a proviral role in Mm-NE cells, contrasting with findings in other bat species. We also identified a potential involvement of Dicer in the cellular stress response. Ongoing work aims to elucidate the mechanisms driving this relocalization and its relevance for Dicer's activity.

Gaucherand L et. al. BioRxiv, doi: https://doi.org/10.1101/2025.07.10.664130

Poster 74

Optimizing site-directed RNA editing of Filamin A as a therapeutic approach for cardiovascular disease, colitis, and vascularized tumors

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Adenosine deaminases acting on RNA (ADARs) deaminate adenosines into inosines in structured RNAs. Since inosines are translated as guanosines, this can lead to recoding events. A highly conserved example of this is the pre-mRNA encoding Filamin A (FLNA), which is endogenously edited by ADARs, leading to a Q-to-R amino acid exchange. Lack of A-to-I editing in FLNA is associated with cardiovascular disease, promotes tumor growth by increasing angiogenesis, and increases susceptibility to developing colitis in both mice and humans. A potential therapy is artificially recruiting endogenous ADARs to induce site-directed RNA editing (SDRE), thereby repairing genetic information. Therefore, I aim to optimize therapeutic SDRE of FLNA to artificially increase editing in endogenous FLNA pre-mRNA and mRNA, thus potentially providing relief to cardiovascular disease, colitis, and cancer patients. This can be achieved with guide RNAs (gRNAs) that form a double-stranded structure where SDRE shall be introduced. However, gRNAs need to be carefully designed – characteristics such as length, nucleotide sequence, and chemical modifications can heavily impact on- and off-target effects, gRNA stability and intracellular delivery, all of which critically affect SDRE. After testing a set of linear gRNAs targeting FLNA and differing in length and chemical modifications, I identified optimal gRNAs that successfully promote targeted A-to-I editing in a reporter-based system as well as in endogenous FLNA of mouse cells. These gRNAs are currently being tested in a mouse model, but this requires troubleshooting. Additionally, an innovative reporter-based screen is being employed that allows the selection of nucleotide sequence-optimized gRNAs.

Poster 75

The m^5C RNA methyltransferase Nsun6 regulates neurodevelopment in mammalian and Drosophila models

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RNA modifications have emerged as critical regulators of gene expression. Among these modifications, RNA 5-methylcytosine (m^5C) is one of the most evolutionarily conserved, being present across a wide range of RNA species. Functionally, m^5C has been implicated in various molecular processes, including mRNA export, RNA stability, and translation regulation. The deposition of m^5C is primarily catalyzed by the NOP2/Sun RNA methyltransferase (NSUN) family. Notably, NSUN6 plays a significant role due to its capacity to methylate both mRNA and specific tRNAs. In our study, we identified deleterious mutations in NSUN6 in three unrelated human patients, each associated with neurodevelopmental disorders and cognitive impairment. To further investigate the functional impact of NSUN6, we utilized a mouse embryonic stem cell (ESC) model and showed that upon differentiation into neural progenitor cells, Nsun6 knockout (KO) cells exhibited neural projection defects. To assess functional conservation, we generated Nsun6 KO models in Drosophila melanogaster. Mutants displayed significant phenotypic abnormalities, including larval motility impairments, neuromuscular junction defects and learning deficits in adults. Interestingly, the tRNA targets identified in flies do not completely overlap with those in mammals, suggesting species-specific substrate preferences. Collectively, these findings highlight a conserved and essential role of NSUN6 in neurodevelopment.

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Poster 76

The role of Gigyf1 in pancreatic β-cell signaling

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Recent whole-exome sequencing studies have linked GIGYF1 loss-of-function to adverse metabolic health, including an increased risk of type 2 diabetes (T2D), elevated blood glucose levels, higher fat mass, and reduced serum IGF1 concentrations. Proper pancreatic β-cell function—through insulin production and glucose-dependent secretion—is critical for T2D pathogenesis. Despite strong genetic evidence, little is known about the roles of Gigyf1 and Gigyf2 in β-cell physiology.
Early studies identified Gigyf1/2 as Grb10 interactors involved in IGF1/insulin receptor signaling. Later, a distinct function emerged: Gigyf proteins regulate translational repression and mRNA degradation via the nonsense-mediated decay pathway.
To investigate the role of Gigyf proteins in β-cell function, we generated Gigyf1/2 single and double-knockout (dKO) cell lines from the rat insulinoma line INS-1E, and genetic mouse models lacking Gigyf1, Gigyf2, or both homologs in the pancreas. These models underwent RNA sequencing and proteomic analysis, revealing Gigyf target genes essential for insulin secretion and β-cell function. Among these were K^+ and Ca^{2+} channel genes, key for insulin secretion activation and membrane repolarization. Consistently, glucose-stimulated insulin secretion (GSIS) was markedly reduced in Gigyf KO islets, while glucagon secretion was elevated. Intracellular insulin content remained unchanged; however, transmission electron microspcopy (TEM) analysis showed fewer insulin granules docked at the plasma membrane, reducing the readily releasable pool.
Interestingly, high fat diet exacerbated the impaired pancreatic function phenotype of GigyfKO mice. Ongoing research aims to integrate the above findings and to elucidate the interplay of metabolic stress and GIGYF1/2 function for insulin secretion and the development of type 2 diabetes.

Poster 77

Targeting the transcription system of poxviruses for antiviral drug discovery

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Poxviruses belong to a large family of double-stranded DNA viruses with a proneness to cause zoonotic infections. They are present in animal reservoirs and responsible for a large spectrum of disorders, including human smallpox and Mpox. To combat these risks imposed by poxviral reservoirs, we outline a structure-based drug design strategy targeting the unique poxviral transcription machinery (vRNAP). We have established a high-throughput screen using fluorescence cross-correlation spectroscopy (FCCS) to identify and design small molecules that interfere with poxviral gene expression, aiming to convert these into specific antiviral drugs.

Poster 78

A Self-supervised Morphological Atlas from Fluorescence Microscopy reveals Astrocyte Plasticity in Amyotrophic Lateral Sclerosis

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Despite decades of research, effective treatments for amyotrophic lateral sclerosis (ALS) remain elusive. Astrocyte reactivity is increasingly recognized as a key driver of neurodegenerative disease progression, including in ALS. However, difficulties in identifying and categorizing reactive astrocyte sub-states hinder a deeper understanding of their roles in pathology and the development of targeted interventions.

Here, we generated a high-content fluorescence microscopy dataset of iPSC-derived astrocytes, both control and ALS-related VCP mutants, cultured under basal conditions or exposed to pro-inflammatory factors (IL-1α, TNF, C1q), and labeled with DAPI and GFAP. We next developed a segmentation-free deep learning framework that learns rich representations from these images, capturing biologically meaningful variation linked to both disease and reactivity. Using these representations, we constructed a morphological atlas of astrocyte sub-states, revealing substantial heterogeneity across and within conditions. By leveraging optimal transport to infer transition trajectories, we further uncovered convergences and divergences between ALS- and inflammation-associated morphologies.

Together, our results present a scalable, annotation-free framework for quantifying astrocyte heterogeneity and identifying disease-relevant morphological signatures, offering new insights into astrocyte dynamics in ALS and other neurodegenerative diseases.

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Poster 79

Transcriptomic characterization of a novel iPSC reference cell line for single molecule mRNA imaging

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Induced pluripotent stem cells (iPSCs) provide a powerful platform for modeling human neurological disorders. Here, we present a transcriptomic characterization of the new reference iPSC line KOLF2.1J, alongside disease-relevant mutant derivatives. These include variants associated with white matter vanishing disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and mutations that impact stress granule dynamics in neurons.
Using ribosome profiling (Ribo-Seq)and mRNA sequencing (mRNA-Seq), we systematically examine gene expression and translational regulation in wild-type and mutant iPSCs. This dataset will establish a foundational map of transcriptomic features in KOLF2.1J and will highlight disease-relevant dysregulation at the RNA level.
Future work will focus on differentiating these lines into mature neurons and applying single-molecule mRNA imaging to directly observe RNA localization, dynamics, and stress granule behavior. Together, this approach aims to provide new insights into how disease-associated mutations alter RNA regulation in the context of neuronal health and neurodegeneration

Poster 80

Modeling human Argonaute-1 mutations associated with intellectual disability in *Drosophila melanogaster*

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Argonaute proteins, core components of the RNA-induced silencing complex (RISC), are pivotal regulators of post-transcriptional gene expression. Among them, AGO1 plays a central role in microRNA (miRNA) biogenesis and function. De novo mutations in AGO1 have been implicated in neurodevelopmental disorders (NDDs) with intellectual disability, collectively termed Argonaute syndromes. Two recurrent variants, Phe180del and Gly199Ser, affect evolutionarily conserved residues, suggesting critical functional importance. However, the mechanisms by which these mutations disrupt AGO1 activity remain unclear.

To elucidate their effects, we modeled the patient-derived variants in Drosophila melanogaster using two strategies: (1) CRISPR/Cas9-mediated editing of the endogenous dAgo1 locus, and (2) generation of UAS-based transgenic lines expressing mutant alleles. To evaluate dAgo1 functionality, we employed a mir-7 overexpression sensor in the patched territory of the fly wing, a sensitive reporter of AGO1 activity. The heterozygous G199S mutation produced a more pronounced phenotype than the heterozygous dAgo1 null allele, suggesting a dominant-negative effect.

These Drosophila models provide a powerful experimental platform to dissect the molecular and cellular consequences of AGO1 mutations and their broader impact on organismal phenotypes.

Poster 81

Improved methods for translation site imaging and visualization of single RNA-binding proteins in live cells

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Single-molecule imaging of translation sites in living cells has enabled the dynamics of protein synthesis to be investigated with high spatial and temporal resolution. These methodologies utilize the interaction between a multimerized epitope tag and its cognate fluorescent nanobody to detect nascent polypeptides as they emerge from the ribosome. In a systematic comparison of current methodologies, we determined that the ALFA-tag Spaghetti Monster (SpaMon) [1] construct reduces perturbations of mRNA expression and increases the fluorescent signal of translation sites [2].

Given the high brightness of the ALFA-tag SpaMon, we next tested whether it could be used for single-protein tracking in live cells. To improve detectability, we immobilized target transcripts at the plasma membrane using a CAAX-based anchor combined with tethering via the 4×ʎN22 peptide and BoxB stem loops [3]. This configuration restricted the imaging volume to the cell cortex, enabling total internal reflection fluorescence (TIRF) microscopy and substantially reducing background fluorescence.

Using PCP-PP7 [4] as a positive control, we validated single-protein visualization and observed expected increases in spot intensity and dwell time with additional PP7 stem loops. Notably, individual PCP molecules displayed an in vivo residence time of ~3 seconds, markedly shorter than predicted from low-nanomolar in vitro dissociation constants [5], underscoring the importance of measuring binding kinetics directly in cells.

This framework enables targeted labeling of translation factors and other regulatory RNA-binding proteins, opening new opportunities to monitor their dynamic interplay with mRNAs and to interrogate how translation is modulated across diverse cellular conditions.

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Poster 82

Linking translation dynamics and protein homeostasis in human degenerative disease

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The mcm^5s^2 modification of wobble uridine (U_{34}) in the anticodon of tRNAs plays, a crucial role in tuning the decoding kinetics of specific codons. This modification is generated by a complex enzymatic network involving the CTU1/CTU2 complex and the Elongator complex, which is composed of ELP1-6. We have demonstrated that a lack of $mcm^5s^2U_{34}$ leads to a loss of proteostasis and the formation of protein aggregates [1], two hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), which are characterized by the accumulation of TAR DNA-binding protein 43 (TDP43) aggregates. Interestingly, potential pathogenic SNPs have been identified within the genomic loci of ELP2 and ELP3 in ALS and FTLD patients [2][3]. However, the mechanistic relationship between $mcm^5s^2U_{34}$ and TDP43 proteinopathies remains unclear.
To dissect the mechanistic relationship between TDP43 and $mcm^5s^2U_{34}$, we use an ALS/FTLD-specific disease model based on HEK293T cells expressing TDP43 variants in an inducible manner [4]. Upon genetic ablation of *CTU2*, *ELP4*,or *ELP5*, we observed accelerated aggregation dynamics of aggregation-prone TDP43 variants, but not of wild-type TDP43. Regular aggregation dynamics can be restored by re-expression of the depleted factors. Interestingly, loss of $mcm^5s^2U_{34}$promotes both nuclear and cytosolic TDP43 aggregation. Conversely, our RNAseq data shows that the expression of TDP43-variants causes a strong deregulation of *ELP1*. Analysis of alternative splicing revealed aberrant splicing of several members of the $mcm^5s^2U_{34}$ pathway including *ELP1*. Collectively, our findings suggest an interdependent relationship in which aggregation-prone TDP43 variants affect the $mcm^5s^2U_{34}$ pathway, and reduced $mcm^5s^2U_{34}$ levels promote TDP43 aggregation.

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Poster 83

RNA decay and KSHV infection: Deciphering the viral-host arms race to control RNA stability

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Rapid and widespread turnover of host mRNA is a cornerstone of infection by the gamma herpesvirus Kaposi's Sarcoma Associated Herpesvirus (KSHV). This process is driven by the viral endoribonuclease SOX, and results in a catastrophic RNA decay event where 70% of total mRNA is lost. This widespread remodeling of the cytoplasmic mRNA landscape causes long range secondary effects, including a sudden loss of mRNA targets for many RNA binding proteins as well as a global hyperadenylation defect in the nucleus and subsequent nuclear export block. While the vast majority of the transcriptome is susceptible to SOX, a subset of host transcripts has been identified as being refractory to this virally-mediated decay event and are efficiently translated into proteins. We showed that these spared transcripts encode a 200nt cis-acting RNA element in their 3'UTR that provides resistance to SOX cleavage. However, while we know how these spared transcripts escape SOX cleavage, we have yet to understand how they escape the nuclear export block associated with SOX-mediated decay. We discovered that these SOX-resistant transcript are hyperadenylated like other transcripts. Intriguingly, these transcripts are still able to be exported out of the nucleus despite carrying these aberrant polyA tails and the longer polyA is maintained in the cytoplasm. Through this work we hope to gain insight into how virally induced alterations to mRNA influences mRNA fate, and to better understand how the dynamics of viral-host interactions alter gene expression.

Poster 84

Maternal age modulates early embryonic translation to influence developmental competence in zebrafish

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Age-related decline in egg quality is one of the main factors contributing to the infertility associated with advanced maternal age. Although the molecular environment of the aged egg is well-characterized, it remains unknown how it translates to the early embryo. In addition to genetic material, the maternal contribution to the embryo encompasses biomolecules (RNAs, proteins, metabolites) and organelles, that are necessary for the gene regulatory events that drive the oocyte-to-embryo transition (OET). Here, we aim to elucidate how maternal age affects molecular processes during the OET and developmental competence of the early embryo using zebrafish as a model. To this end, we integrated multi-omics approaches and uncovered that the proteome of the early embryo is perturbed as a result of advanced maternal age, despite minor changes at the mRNA level. We identified a global, transient upregulation of translation levels as a potential culprit behind increased protein levels in the embryos from old mothers. Increased levels of translation were observed as early as one hour post-fertilization and persisted until three-hours post-fertilization, coinciding with increased lethality during the first three hours of development in embryos from aged mothers. Interestingly, although mild translation inhibition caused embryonic lethality in offspring from young mothers, the offspring from their aged counterparts were found to be resistant, suggesting that increased translation allows for buffering against translational stress. Taken together, our data point towards translation as a central gene regulatory process influencing developmental competence of early embryos in response to advanced maternal age.

Poster 85

Enhancer lncRNA LOC730338 modulates BCR signaling and immune evasion in lymphoma by regulating RNA homeostasis

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Chronic antigenic stimulation is a central factor in the development of marginal zone lymphoma (MZL). While the pharmacological inhibition of the B-cell receptor (BCR) signaling by Bruton's tyrosine kinase (BTK) inhibitors is initially effective, the development of resistance remains a challenge in treating MZL and other B-cell malignancies [1]. Enhancer activation remodeling is a key epigenetic mechanism that enables tumor adaptation during therapy. The most active regulatory regions cluster in super-enhancers and produce enhancer RNAs (eRNAs), a class of unstable noncoding transcripts that primarily serve as scaffolds for chromatin looping. However, when stabilized, these eRNAs can evolve into long noncoding RNA (lncRNAs) with distinct functions [2].
To investigate enhancer-associated long non-coding RNAs (elncRNAs) involved in shaping BCR pathway dependence, we conducted a CRISPR interference (CRISPRi) screen in MZL cells [3]. We identified LOC730338, an elncRNA linked to A-to-I RNA editing, which we renamed ADARreg [4]. ADARreg renders tumor cells refractory to BCR pathway inhibition by modulating ADAR2 nuclear translocation and altering RNA modification patterns in key regulatory isoforms through coordinated control of RNA stability and localization, as uncovered by direct RNA sequencing performed in subcellular compartments [5]. In addition, ADARreg induces an immune-suppressive transcriptional program, increasing the production of inhibitory cytokines and receptors that diminish NK cell-mediated cytotoxicity [6].
Together, these findings uncover a novel role for elncRNAs in orchestrating immune evasion and provide a potential therapeutic strategy to overcome resistance in lymphoma and other immune-related diseases.

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Poster 86

UPF1 shuttles between nucleus and cytoplasm independently of its RNA-binding and ATPase activities

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The ATP-dependent RNA helicase Up-frameshift 1 (UPF1) is an essential protein in mammalian cells and a key factor in nonsense-mediated mRNA decay (NMD), a translation-dependent mRNA surveillance process. UPF1 is mainly cytoplasmic at steady state but accumulates in the nucleus after inhibiting CRM1-mediated nuclear export by Leptomycin B (LMB), indicating that UPF1 shuttles between the nucleus and the cytoplasm. Consistent with its dual localization, there is evidence for nuclear functions of UPF1, for instance in DNA replication, DNA damage response, and telomere maintenance. However, whether any of UPF1's biochemical activities are required for its nuclear-cytoplasmic shuttling remains unclear.
To investigate this, we examined two UPF1 mutants: the well-described ATPase-deficient UPF1-DE (D636A/E637A) and a newly generated RNA-binding mutant UPF1-NKR (N524A/K547A/R843A). Biochemical assays confirmed that the UPF1-NKR mutant cannot bind RNA or hydrolyze ATP *in vitro* but retains interaction with UPF2 and UPF3B. Overexpression of UPF1-NKR exerted a dominant-negative effect on endogenous UPF1 and inhibited NMD. Subcellular localization studies revealed that UPF1-DE accumulates in cytoplasmic granules (P-bodies), even in the presence of LMB, whereas UPF1-NKR shuttles normally. This indicates that UPF1's shuttling does not require its RNA-binding or ATPase activities. Notably, the UPF1-DE-NKR double mutant restored nuclear-cytoplasmic shuttling and prevented accumulation in P-bodies, suggesting that the shuttling defect of UPF1-DE arises from its tight binding to RNA. Overall, our findings demonstrate that UPF1's shuttling is independent of its ATPase and RNA-binding activities, with RNA binding itself being a key determinant of its cytoplasmic retention.

Poster 87

The m^{6}A modification and translational adaptation

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Transfer RNAs (tRNAs) are among the most heavily modified molecules, with more than ten different types of modifications present at position 37 in the anticodon stem loop [1]. One of these modifications is N^6-methyladenosine (m^{6}A), found only in tRNA$_1^{Val}$ in *Escherichia coli*. This modification is introduced by the enzyme TrmM at the A37 residue within an ACA motif. It is shown that deletion of *trmM* gene does not significantly impact cell growth under physiological conditions, but the modification that it is introducing can be advantageous during stress [2].
Previous studies from our lab showed that when bacteria encounter diverse stress conditions, the sequence-specific endoribonuclease MazF becomes active and cleaves single-strand RNAs by ACA sites, among which tRNA$_1^{Val}$ molecules [3]. MazF cleavage leads to generation of tRNA$_1^{Val}$ halves, with still unknown, but possible function in bacterial gene expression, maybe even as a part of a broader regulatory network. Hence, m^{6}A37 modification may also serve as a regulatory element in tRNA$_1^{Val}$ cleavage, because when present, MazF cleavage is prevented.
With the focus on the m^{6}A modification, the goal of my project is to understand how tRNA modifications influence translation dynamics in *Escherichia coli* and determine the environmental conditions that modulate its presence. To assess the stability and translational efficiency of modified and non-modified tRNA$_1^{Val}$, specific reporter molecules will be used, designed to contain only one kind of valine codon, allowing us to gain closer insight into the codon usage and translation dynamics.

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Poster 88

Antisense oligomers mitigate the genotoxic activity of colibactin-producing *Escherichia coli*

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Although antisense oligomers (ASOs) have primarily been used to regulate gene expression in eukaryotic systems, recent advancements have highlighted their potential for application in bacteria. So far, their development has focused on antimicrobial strategies targeting essential bacterial genes to kill bacterial pathogens. However, their potential to selectively suppress harmful traits in commensal bacteria without eradicating them remains largely unexplored. Here, we investigate the use of ASOs to inhibit the production of the genotoxin colibactin in a clinical *Escherichia coli* isolate.
Colibactin is a secondary metabolite produced by several Enterobacteriaceae. Its genotoxic activity has been linked to the development and progression of colorectal cancer. Colibactin is encoded by a gene cluster, referred to as *pks* (polyketide synthase) island, that comprises 19 *clb* genes (*clbA*-*clbS*). We designed peptide nucleic acid (PNA)- based ASOs targeting different mRNAs from the *pks* island. These ASOs effectively blocked target protein synthesis in the colorectal cancer isolate *E. coli* CCR20 *pks*+. Among these, *clbR*-targeting ASOs effectively reduced colibactin-induced DNA damage in infected HeLa cells, indicating that clbR is a particularly effective target to disrupt colibactin production.
Together, our findings demonstrate targeted inhibition of colibactin biosynthesis and associated genotoxicity via antisense knockdown. Our approach highlights the potential of ASOs as a strategy to selectively modulate unwanted gene expression in otherwise benign members of the human microbiome, extending their application beyond antimicrobial use.

Poster 89

High-pressure NMR reveals stress-induced changes in multicomponent biomolecular condensates

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Liquid–liquid phase separation (LLPS) allows proteins and RNAs to form membraneless assemblies known as biomolecular condensates. These structures support cellular organization and regulation, but when LLPS is disrupted, condensates can gradually harden into solid-like aggregates and fibrils. Such aberrant phase transitions, particularly involving proteins like FUS and TDP-43 or repeat-expansion RNAs, are closely linked to neurodegenerative diseases.

To study these aberrant phase transitions and maturation pathways, we use nuclear magnetic resonance (NMR) under high hydrostatic pressure. Pressure shifts biomolecules toward low-volume, high-energy conformations, acting as a lens that reveals hidden molecular states. Our data show that FUS undergoes an unexpected transition into a distinct high-pressure condensate state above ~2000 bar, with altered condensate properties and internal protein dynamics. In aged FUS samples, prolonged pressure exposure dissolves pre-formed fibrils, coinciding with destabilization of the Zinc-finger domain. This implies that structural destabilization drives redistribution of protein populations and fibril melting, offering a controlled way to probe condensate maturation and aging.

To understand which specific biomolecules participate in these transitions in the context of multicomponent condensates, we developed in addition a non-invasive multiplexed NMR filter.1 This can simultaneously differentiate several proteins/RNAs within the same condensate without any label required. By exploiting diffusion and relaxation differences between molecules inside condensed droplets and the dilute phase, this method separates signals from multiple protein populations, even when NMR signals are completely overlapped.

Together, pressure-based perturbation and multiplexed NMR provide a strategy for dissecting how multicomponent condensates assemble, adapt to stress, and mature toward disease-associated states.

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Poster 90

A role for mRNA translation control in oxytocinergic signaling and social behavior adaptations

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Translational regulation plays a key role in neuronal plasticity [1,2]. However, the pivotal targets of how regulated translation is leveraged for neuronal adaptations are poorly understood. I am testing the hypothesis that *oxytocin receptor (Oxtr)* transcript isoforms are subject to differential regulation of translation, thus, directing neuronal plasticity for behavioral adaptations. The oxytocinergic system regulates key elements of social interactions, recognition and reward [3] and there is evidence that the expression of the OXTR, a G-protein coupled receptor (GPCR), is dynamically regulated [4,5]. Using long- and short-read sequencing methods we identified three transcript isoforms for the mouse *Oxtr* gene. Interestingly, two of the resulting *Oxtr* transcript isoforms contain upstream open reading frames (uORFs) that recruit ribosomes in their 5' UTRs and repress translation from the coding open reading frame *in vitro*. To test the role of these uORFs in OXTR regulation *in vivo*, we generated knock-in mice with point-mutations in the uORF start codons ($Oxtr^{ex1a\Delta uATG}$). Strikingly, these $Oxtr^{ex1a\Delta uATG}$ mice show a decrease in OXTR protein levels compared to WT mice. Moreover, $Oxtr^{ex1a\Delta uATG}$ mice exhibit an altered behavioral response in a social recognition assay. This work uncovers a novel post-transcriptional mechanism controlling OXTR levels and social behavior and highlights the importance of alternative transcript isoforms in neuronal plasticity.

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Poster 91

Exploring roles of TnpB beyond transposition control in *Helicobacter pylori*

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The CRISPR-Cas nuclease Cas12 is one of the primary tools of the new generation of genome-editing methods. Cas12 was found to have evolved from the transposon-encoded nuclease TnpB, which acts as an RNA-guided homing endonuclease in bacteria. To drive transposon maintenance, TnpB mediates double-strand DNA breaks via a guide RNA called ωRNA, which originates from the *tnpB* 3' end [1]. Recently, nuclease-dead TnpB homologs from Enterobacteriaceae were surprisingly shown to act as RNA-guided transcription factors [2]. Here, we aimed to explore whether catalytically active TnpB homologs might also be repurposed for gene regulation using the gastric pathogen *Helicobacter pylori* as a model. Our previous and unpublished differential-RNA sequencing data indicated the presence of ωRNAs in five TnpB-encoding loci of *H. pylori* strains 26695 and G27 [3], of which we could validate expression for eight out of ten loci using Northern blotting. Moreover, a previous *H. pylori* transposon mutagenesis screen indicated that *tnpB* might impact mice colonization [4]. To further explore the potential role of TnpB in virulence, we constructed TnpB deletion mutants, which we will use for *in-vitro* infection assays and RNA-seq based transcriptome analysis. Northern blots of the deletion mutants showed loss of ωRNA expression, while partial *tnpB* mutants retained low ωRNA expression. Preliminary SDS-PAGE analysis revealed several deregulated protein bands upon *tnpB* deletion, which we are currently analyzing. Overall, our study aims to provide additional, fundamental insight into the alternative roles of TnpB proteins beyond transposition control, which could inspire the development of new biotechnological tools.

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Poster 92

Synthetic mRNA Platform

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The mRNA Platform Zurich aims to design, optimize, produce and distribute in vitro transcribed (ivt) synthetic messenger RNA (mRNA). Synthetic mRNA has been a key component in the COVID-19 vaccines authorized worldwide, including BioNTech/Pfizer's Comirnaty, Moderna's Spikevax, and Arcturus' Kostaive. Ongoing research suggests its potential in the prevention and treatment of diverse diseases (e.g. degenerative diseases, cancer, autoimmune disorders and infections). Our goal is to adapt mRNA to specific applications by optimizing its functionalities. To achieve this, we refine its sequence (in untranslated and translated regions), explore new modifications (allowing better stability, enhanced translation or controlling immunostimulating activities) and test different mRNA formats (linear, circular, replicating). Our main purpose is to promote the use of synthetic mRNA technology for therapeutic and scientific applications.

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Poster 93

The role of Nonsense-mediated mRNA decay in early mammalian development

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Embryonic development is a dynamic but tightly regulated process that depends on the precise expression and turnover of mRNA molecules. While transcriptional control has traditionally been viewed as central to developmental gene regulation, post-transcriptional mechanisms, including mRNA decay pathways, are increasingly recognized as important contributors to embryonic development. Nonsense-mediated mRNA decay (NMD), a conserved mRNA surveillance pathway first described for its role in removing transcripts with premature termination codons, has also been shown to regulate a broad set of physiological mRNAs. Many of these transcripts are associated with developmental processes, yet the exact role of NMD during early mammalian development is still largely unknown.
Here we aim to define the roles of NMD during early mammalian development by determining when, where, and in which specific cell types NMD activity influences developmental gene expression programs. Using gastruloids derived from mouse embryonic stem cells (mESCs), we aim to examine how NMD affects early cell fate decisions and the expression of transcripts relevant for germ layer specification. Using a targeted CRISPR Cas9 knockout screen and pharmacological inhibition of NMD, combined with single-cell RNA sequencing and proteomics, we aim at providing novel insights into the regulatory functions of NMD during early embryonic development and identifying molecular pathways that depend on proper NMD activity.

Poster 94

Pooled CRISPR Screens identify a lncRNA involved in the innate inflammatory Immune Response

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Cardiac resident macrophages (CRMs) have recently gained recognition as a pivotal cell population in the heart, playing key roles in cardiovascular health and disease. Yet, the precise functions of CRMs remain poorly understood. While long non-coding RNAs (lncRNAs) have been recognized for their role in resident macrophages in various organs, no functional analysis of lncRNAs in CRMs has been reported.
This study aims to elucidate the functional significance of lncRNAs in CRMs in health and disease. We selected 30 CRM-enriched, disease-regulated, and human-conserved lncRNAs and investigated them simultaneously using CRISPR-droplet sequencing (CROP-Seq), a pooled CRISPR screen linking lncRNA perturbations to transcriptomic phenotypes at single-cell resolution.
Across three CROP-Seq experiments, we identified seven candidates with clear transcriptomic phenotypes. Among them, the lncRNA NIP16 showed a striking regulatory effect on a cluster of Clec4 pattern recognition receptors (PRRs), which detect microbial and damage-associated glycans triggering immune activation. RNA-seq analysis of NIP16 knockdown macrophages confirmed the pronounced deregulation of Clec4 genes alongside other immune-relevant pathways. Following stimulation with a mycobacterial-derived CLEC4D ligand, NIP16-deficient macrophages exhibited a markedly impaired inflammatory response. Its potential involvement in microbial and sterile inflammation will be investigated using knock-out mice and disease models. Given NIP16's conservation in human, its therapeutic potential is currently assessed in hiPSC-derived macrophages.
In summary, we established a high-throughput CROP-Seq workflow revealing seven functionally relevant lncRNAs. NIP16 emerged as a key regulator of PRR-mediated inflammatory signalling and will be investigated further in vivo and in hiPSC-derived macrophages to evaluate its relevance for cardiovascular disease.

Poster 95

High throughput protein crystallization platform

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The High Throughput Protein Crystallization facility provides the opportunity to any NCCR RNA & Disease group to complement their projects with X-ray crystallographic analysis. The facility offers the possibility to set up high throughput crystallization experiments at nano-liter scale at 4 °C or 20 °C, screening up to 20'000 conditions per day.
Potential users can contact the platform scientists any time for advice on protein sample preparation and choice of initial screens. The experiments can be monitored remotely by the users using two Rock Maker incubation and imaging systems, and the platform scientists support the users with advice on follow up of initial crystallization hits. We offer the possibility of harvesting and testing initial crystal hits at the Swiss Light Source synchrotron. Once the initial conditions are identified, the Jinek group can work with the users to optimize the crystals, collect data and solve the structure on a collaborative basis.
If your group has a project that could be strengthened with structural information, please contact the platform (rna.xtal@bioc.uzh.ch). We will be happy to help you to obtain an atomic model of your protein or complex.

Poster 96

Towards covalent fluorescent light-up aptamers (coFLAP) for RNA imaging

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Fluorescent light-up aptamers (FLAPs) are RNAs that conditionally bind small-molecule dyes and induce their fluorescence, enabling imaging of RNA in living cells [1]. Recently, the first covalent FLAP was reported for the Pepper–HBC system [2]. Converting a noncovalent FLAP into a covalent FLAP (coFLAP) offers unprecedented potential to improve signal-to-noise ratios: a covalent tether between aptamer and fluorophore withstands washing steps that reduce nonspecific fluorescence from excess dye. A further advantage of coFLAPs is the ability to perform fluorescence recovery after photobleaching (FRAP) experiments, because covalent tethering prevents dynamic exchange of bleached dye with unbleached dye. As a result, measurements of localization kinetics for coFLAP-labeled target RNAs become feasible, underscoring the value of expanding the coFLAP toolkit. Here, we present ongoing work toward a covalent version of the recently described Clivia–NBSI FLAP [3]. Guided by the crystal structure of the noncovalent Clivia–NBSI complex [4], we are designing ligands bearing an electrophilic handle to enable proximity-induced covalent tethering. Our goal is to broaden the utility of FLAPs as biotechnological tools for RNA localization in living cells, thereby advancing our understanding of RNA dynamics and function. Moreover, these studies contribute to new strategies for covalent cellular RNA labeling and have implications for RNA–small-molecule drug targeting.

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Poster 97

The eIF3-Gpbp1 axis: a paradigm for eIF3-mediated repression?

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Protein synthesis is a multi-step process known to be regulated primarily at the initiation stage. Translation initiation is largely dictated by the dynamic recognition and interplay of structured elements embedded in mRNA 5'UTRs with RNA-binding proteins, RNA helicases, and the translation machinery. While the regulatory roles of many structural elements in mRNA 3'UTRs are well documented, the identities and structure-function relationships of those located in mRNA 5'UTRs remain poorly understood.
Albeit its central role as part of the 43S pre-initiation complex, the multi-subunit eIF3 complex has recently been described to selectively influence the translation of specific mRNAs by recognizing stem-loop structures in their 5'UTRs. Although eIF3-mediated sensing of these structures can either enhance or inhibit translation, most attention has centered on mechanisms of translational activation. Based on recent observations and our identification of the previously unknown eIF3 interactors Gpbp1 and its paralog Gpbp1l1, we hypothesize that specific structural elements may collaborate with eIF3 to repress translation of selected transcripts. Gpbp1 utilizes its N-terminal extended AT-hook to associate with GC-rich sequences in mRNA 5'UTRs. In line with a possible repressive role, both Gpbp1 and its paralog additionally engage with the Ccr4-Not complex via their C-terminal vasculin-like domains. By using Ribo-Seq, we are currently investigating whether Gpbp1 is part of an eIF3-containing repressor complex, which modulates mRNA translation. Following validation of Gpbp1 as a repressor and definition of a high-confidence set of regulated mRNAs, we aim to employ unbiased approaches to uncover additional components required for eIF3-mediated repression.

Poster 98

Structural characterisation of RNA-Edited FLNA/FLNB Ig22 Domains

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Adenosine-to-inosine (A-to-I) modification in mRNA can lead to protein recoding because the translation machinery interprets inosine as guanosine, thereby introducing a new amino acid into the polypeptide chain. Filamin A (FLNA) and Filamin B (FLNB) are among the few proteins outside the nervous system that undergo such recoding, resulting in a glutamine-to-arginine (Q→R) substitution within immunoglobulin (Ig) repeat 22.
In our previous studies, we have shed light on the relationship between A-to-I editing and its physiological function in FLNA. For instance, a significant decrease in FLNA editing was identified in patients associated with cardiovascular diseases. In the follow-up studies, we have shown that editing affects the FLNA mechanical properties and actin cross-linking. The FLNA editing also altered the cellular stiffness, cell migration and cell adhesion. More recently, we have demonstrated FlnA editing modulating vascularisation and tumour growth.
Despite these functional insights, it has remained unclear how the Q→R exchange affects the structure of FLNA and FLNB proteins, and whether this amino-acid substitution alters their binding preferences. To address this gap, we solved the crystal structures of the Ig22 domains of both FLNA and FLNB. Although the Q→R substitution does not introduce major conformational changes, our structural analyses revealed a unique N-terminal segment within Ig22 that distinguishes it from other Ig repeats. NMR spectroscopy further confirmed the presence and architecture of this distinct N-terminal structural element.
The structural and functional implications of these findings will be discussed.

Poster 99

Synthetic strategies for diverse chemical modifications of antisense oligonucleotides

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RNA Therapeutics have gained widespread attention in recent years as a novel therapeutic platform. The remarkable versatility of RNA provides numerous opportunities for their use in medicine. One promising new concept involves antisense oligonucleotides (ASOs) which can be designed to target a specific mRNA sequence. This strategy allows for the regulation of disease-associated protein translation, modifications of splicing patterns or inhibition of gene expression [1]. ASOs can be employed to suppress vital genes in cancer cells [2] or serve as antibiotics [3] by targeting essential genes in bacteria.
Despite the versatility of ASOs in terms of their function and pathways, most of them face significant challenges when it comes to the application of the drugs in vivo. ASOs are large, negatively charged molecules which cannot easily pass through the lipid bilayer into their target cells [4]. As a result, their efficiency largely depends on effective delivery as well as stability in vivo and non-immunogenicity. Overcoming these barriers is essential for maximizing the therapeutic potential of ASOs.
We will present synthetic strategies for new and advanced chemical modifications that can be introduced into oligonucleotides via solid-phase synthesis. These modifications aim to enhance stability, maintain low immunogenicity and improve cellular uptake. Our strategy builds on established ASO modifications, particularly 2'-O-modified phosphoramidites, and combines them with either conjugation chemistry, enabling the simultaneous introduction of diverse new functionalities, or with the direct chemical incorporation of natural modifications. Both approaches allow these features to be strategically positioned within ASO sequences and their impact on biological functions to be evaluated.

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Poster 100

The role of cellular condensate maturation in gene expression

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Biomolecular condensates are ubiquitous and have been implicated in a wide range of biological processes. Several condensates were shown to transition over time from a liquid-like state into solid-like aggregates in a poorly understood process described as aging, hardening or maturation. Condensate maturation into solid-like aggregates has been suggested to play a critical role in many neurodegenerative diseases. However, so far condensate maturation has almost exclusively been studied in vitro under precisely calibrated environments. The aim of this study is to examine the aging of condensates and the functional consequences thereof directly in cells. To this end, we established a human cell model that accumulated dynamically arrested condensates over time. In parallel, we introduced a reporter mRNA into human cells and specifically recruited it into P-bodies, a persistent biomolecular condensate in many cells. We confirmed and quantified this recruitment via single molecule live imaging and found that P-body localization correlates negatively with the expression of the reporter. In order to study the effects of condensate hardening, we introduced modified versions of the P-body protein DDX6 in cells, leading to P-bodies with different material properties, and monitored the effect on the expression of the recruited reporter mRNA.

Poster 101

Mapping sites of RNA oxidation in aged tissues

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Oxidative stress is known to contribute to many aging-associated changes through reactive oxygen species (ROS) [1]. These ROS react with and damage macromolecules such as proteins, lipids, DNA, and RNA [2]. While DNA has been the focus of research into the effects of oxidative damage to nucleotides [2], RNA oxidation is quickly becoming a topic of interest [3,4]. Recent studies under conditions associated with stably heightened levels of ROS such as Alzheimer's [5]. In these conditions we hypothesize that despite the short half-life of RNAs, the cell would experience a constant load of oxidized RNAs. Such an oxidative environment has also been observed in aged cells broadly [1] raising the question of whether RNA oxidation impacts the proteome of aged cells. Our goals are to establish methods for the discovery and analysis of inherent patterns of RNA oxidation, and to assess the impact of RNA oxidation on translation and the proteome. Next, we intend to use in vivo mouse samples, gathered as part of a larger experiment on aging, to compare all these features among tissues of young and old mice.
We focus on 8-oxo-guanine (8oxoG), the most common oxidative lesion in nucleotides [6], as the main indicator of oxidative damage. 8oxoG can bind both cytosine and adenine [7,8] potentially interfere with a variety of cellular processes, including translation and miRNA function, both play important roles in maintaining proteostasis.
We will present the progress on in establishing our methodologies and our observation with regards to smRNA oxidation in cells line models of oxidative stress, focussing on miRNA.

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Poster 102

Impact of tRNA m^{1}G9 methylation in insulin-secreting β-cells

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In Archaea and Eukarya, purines at position 9 of tRNAs are N^1-methylated by members of the TRM10 family of methyltransferases. In human, TRMT10A is the m^{1}G9-specific isoform that methylates a subset of nuclear-encoded tRNAs and its loss-of-function mutations have been linked to neurodevelopmental disorders and early-onset diabetes. This project aims to identify the functional significance of TRMT10A and its involvement in the metabolism of insulin-secreting beta-cells. We investigated the pathophysiological effects of the lack of m^{1}G9 modification in insulin-secreting rat cells.
For this purpose, we generated insulin-secreting rat cells knocked out (KO) for TRMT10A. These cells lacking TRMT10A were subjected to next generation sequencing analysis confirming the absence of the m^{1}G9 modification and revealing an altered steady state level of a number of tRNA targets of the enzyme.
Using ELISA and calcium signaling assays, we found that the TRMT10A-KO cells have a higher insulin secretory activity. Global protein synthesis assay didn't reveal any significant difference at the level of total protein production. However, transfection experiments using an EGFP-based reporter showed deficiency in EGFP fluorescence in the TRMT10A-KO cells, highlighting a protein translation alteration. Additionally, we found that the TRMT10A-KO cells had a higher propensity to produce protein aggregates in response to different stress stimuli, but a lower activation of the ER stress response pathways.
These findings shed light on molecular pathways affected by TRMT10A in this insulin-secreting cell line, and they serve as a foundation for understanding the process underlying the development of diabetes in patients with TRMT10A loss-of-function mutations.

Poster 103

RNA levels determine stress-specific P-body properties

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Translational repression and mRNA degradation are essential mechanisms that allow rapid cellular adaptation to environmental changes. Under specific stresses, mRNA and decay factors accumulate in processing bodies (PBs). PBs are cytoplasmic, membrane-less organelles involved in mRNA degradation and/or storage. The morphology of PBs is shaped by the encountered stress. While PB formation during glucose starvation is well characterized, less is known about their behavior under endomembrane system or redox balance stresses. Here, we showed that PBs exhibit stress-specific differences in number, brightness, dynamics, and core component recruitment in *S. cerevisiae*. The ER stresses DTT and tunicamycin cause mild translation attenuation, induce dim PBs that sequentially recruit core components from the 5' to the 3' UTR and display more viscous, less fluid-like behavior. In contrast, stresses causing stronger translation attenuation induce brighter, more fluid PBs formed by rapid *en bloc* component recruitment. To explore the relationship between translation and PB properties further, we deleted *BFR1* and *SCP160*, two polysome-associated proteins, which shifted DTT-induced PBs towards a brighter phenotype and promoted earlier recruitment of 3' components, like Pat1 and Lsm4. Similarly, depleting Not1, the scaffolding protein of the Ccr4-Not1 deadenylation complex, increased cytoplasmic levels of non-translating mRNA, enhanced the brightness of DTT-induced PBs. Finally, an *in vitro* assay established the correlation between RNA concentration and brightness of Dhh1 droplets, demonstrating that RNA abundance largely dictates PB characteristics.

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Poster 104

The Structured RNA-binding Domains and Condensation Capacity of FUS Shape its RNA-binding Landscape and Function

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Fused in Sarcoma (FUS) is a ubiquitously expressed RNA-binding protein which regulates several aspects of RNA metabolism and contributes to the DNA damage response. Besides two structured RNA-binding domains that mediate interactions with RNA, FUS also comprises intrinsically disordered regions that drive biomolecular condensation. While the molecular basis of these properties is increasingly well-characterised in vitro, it remains unclear how condensation interfaces with sequence-specific RNA recognition in cells to shape the physiological functions of FUS.
Here, we use strategically designed point mutations to selectively impair FUS condensation or canonical RNA-binding and examine their effects upon endogenous expression in U2OS cells. We find that both properties contribute to the organisation of nuclear ribonucleoprotein condensates, and that each shapes distinct aspects of FUS function in the DNA damage response. Transcriptome-wide mapping of FUS-RNA interactions reveals that condensation and canonical RNA-binding modulate binding to different subsets of FUS binding sites, associated with distinct sequence and structural features. Complementary transcriptomic profiling highlights a cooperative interplay between the two properties in regulating FUS-dependent gene expression and alternative splicing.
Together, these findings provide a mechanistic framework for understanding how FUS integrates its condensation capacity with sequence-specific RNA recognition to coordinate nuclear organisation, RNA metabolism, and genome stability. In a wider context, our work suggests that beyond driving partitioning into micron-scale condensates, the condensation propensity of RNA-binding proteins plays a nuanced role in shaping their RNA interactions and regulatory activities.

Poster 105

Mycobacterium abscessus Eis2 is an aminoglycoside resistance determinant and pro-drug activator of ribosomal antibiotics

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Mycobacterium abscessus has the reputation as antibiotic and clinical nightmare due to the multitude of resistance mechanisms and consequently being recalcitrant towards antibiotic treatment. We previously showed that the broad-spectrum *N*-acetyltransferase Eis2 is a *whiB7*-regulated resistance determinant for various ribosomal antibiotics (kanamycin, hygromycin, capreomycin and amikacin). Eis2 increases the minimal inhibitory concentration [1], the minimal bactericidal concentration MBC [2] and negatively affects the treatment outcome of the cornerstone drug amikacin in a pre-clinical model of *M. abscessus* infection [3]. Our recent results demonstrate that the innate resistance determinant Eis2 can be turned against its producer [4]. Florfenicol amine (FF-NH_2), a metabolite of the antibiotic florfenicol, is devoid of ribosome inhibition activity unless Eis2 converts it into florfenicol acetyl (FF-Ac). This creates a feedforward bioactivation loop: as FF-NH_2 is activated, WhiB7 and Eis expression increase, further amplifying antimicrobial activity. In vivo, FF-NH2 shows potent anti-*M. abscessus* activity comparable to current standard of care antibiotics while the Eis2-dependent pro-drug activation mechanism avoids toxic side effects since mitoribosomal protein biosynthesis remains undisturbed. This proof-of-concept study illustrates how intrinsic resistance determinants can be repurposed for selective pro-drug activation, transforming bacterial defense into vulnerability – a promising new paradigm for treating multidrug-resistant (myco-)bacterial infections.

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Poster 106

Characterization of the Not condensates and their role in regulation of translation elongation dynamics

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Ccr4-Not complex is a eukaryotic master regulator of gene expression homeostasis [1,2]. It is a conserved multi-subunit complex built upon central scaffold protein Not1 onto which other subunits dock. Two enzymatic modules that dock on Not1, first is the deadenylase module comprising Caf1 and Ccr4. Second, is the ubiquitination module composed of Not4, a RING E3 ligase. Reports suggest role of the Ccr4-Not complex in co-translational processes, such as co-translational assembly of proteins and delivery of translating mRNAs to destined organelles [3,4]. This is coherent with an interesting structural study showing the association of the complex to elongating ribosomes as a decoding mechanism via Not5 binding to the ribosomal E site [5]. Thereby, the Ccr4-Not complex is empowered with regulatory decisions that determine the fate of the translated protein and their encoding mRNAs. Our goal is centred around the recent findings that Not proteins form condensates and by means, could regulate the translation elongation dynamics for production of functional, well-assembled proteins [6]. Although an increasing plethora of physiological functions linked to Ccr4-Not complex are being discovered, little is known about how these different functions are interconnected and regulated. Moreover, we have very little knowledge about the composition, dynamism and factors that trigger condensate formation in-vivo. Recent studies determined that Not1 and Not4 subunits inversely regulate mRNA solubilities [7], so we cannot assume that Not proteins co-localize in the same condensates. This project aims to characterize the Not protein condensates, their regulation and their link to translation elongation dynamics in the model organism, yeast S cerevisiae.

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Poster 107

Isolation of endothelial extracellular vesicles from plasma as a basis for RNA biomarker discovery in sickle cell disease

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Sickle cell disease (SCD) is one of the most common hereditary diseases, characterized by chronic hemolysis, inflammation, and recurrent vaso-occlusive episodes. A key feature of SCD is progressive systemic vasculopathy, caused by ongoing endothelial activation and injury [1,2]. Endothelial-derived extracellular vesicles (EVs) have gained attention as potential contributors to this process. As promising biomarkers, they carry bioactive molecules, including RNAs that may reflect endothelial activation and damage [3,4]. Identifying endothelial EV-associated RNA signatures could thus provide minimally invasive indicators of endothelial damage and vascular pathology in SCD. However, the selective enrichment of endothelial-specific EVs from human plasma remains challenging and requires robust methodological standardization.
This project aims to develop a workflow for isolating endothelial-derived EVs from human plasma to facilitate downstream RNA analyses. The protocol includes an initial EV enrichment step followed by immunoaffinity isolation using the endothelial surface marker CD144. The isolated EVs are evaluated using nanoparticle tracking analysis (NTA) to determine particle concentration and size distribution, and RNA is extracted from CD144-positive EV fractions to assess their suitability for future transcriptomic studies.
Preliminary results show that the workflow is effective and reproducible. Endothelial EVs can be enriched reliably, producing clear NTA signals and detectable RNA. IgG controls indicate minimal background, supporting the approach's specificity. Ongoing optimization aims to improve EV and RNA yield. Once fully developed, this workflow will lay the groundwork for future studies exploring RNA-based endothelial EV biomarkers to better understand vascular injury in SCD.

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Poster 108

Condensation of repeat-expansion RNA requires dynamic RNA remodeling that is chaperoned by muscleblind-like splicing regulators

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Accumulation of repeat expansion RNA in nuclear RNA foci is a hallmark of repeat expansion disorders [1]. RNA can undergo reversible phase separation in vitro above a lower critical solution temperature (LCST), yet the molecular basis for RNA LCST-type phase transitions remains poorly understood [2]. Accordingly, it is unclear whether neurotoxic RNA foci are formed by similar RNA phase transitions. Using CAG- and CUG-repeat expansion RNA as model systems, we show that RNA phase separation strongly promotes folding within the condensates. We provide a mechanistic basis for RNA LCST-type phase transitions, in which thermally induced conformational dynamics nucleate RNA droplet formation above a threshold temperature. Accordingly, the LCST depends on the overall RNA-RNA interaction strength in solution. We rationalize this with an extended Flory-Huggins model coupled to a two-state transition for temperature-induced RNA dynamics. Increasing the number of trinucleotide repeats promotes condensation due to a less folded ground state. Using our recently developed condensate detection by semi-solid magnetization transfer (CONDENSE-MT) method [3], we further demonstrate that CAG-RNA condensates are gel-like, semi-solid materials that contain less water when increasing the number of repeats. While RNA-only condensate formation requires elevated temperatures, we show that the protein muscleblind-like splicing regulator 1, which is colocalized heavily with pathological RNA foci [4], acts as an RNA chaperone [5] for the formation of highly folded RNA condensates at physiological temperatures. Our work identified a new, RNA-driven class of biomolecular condensate and suggests that RNA LCST-type phase transitions, modulated by disease-associated proteins, underlie the formation of pathological RNA foci in repeat-expansion disorders.

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Poster 109

A human cell-free translation screen identifies the NT-2 mycotoxin as a ribosomal peptidyl transferase inhibitor

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Translation inhibitors are invaluable for probing ribosome function and therapeutic applications, but systematic discovery in human systems is limited by the lack of scalable, screening-compatible cell-free platforms. Here, we establish a robust high-throughput screening using human lysates that bypasses cellular cytotoxic effects. After screening ~28,000 small molecules, we identified known and novel translation inhibitors, including NT-2, a trichothecene mycotoxin produced by the pathogenic Fusarium sporotrichioides. NT-2 suppressed protein synthesis in human cells and yeast lysates, while sparing translation in bacteria and intact yeast cells. Cryo-EM at 1.72 Å revealed NT-2 bound at the peptidyl transferase center of the human 60S ribosome, confirming NT-2 as a eukaryote-specific elongation inhibitor that engages ribosomes in a dormant, SERBP1-bound state. Together, these results expose NT-2 as a previously unrecognized environmental inhibitor of mammalian protein synthesis and demonstrate the power of cell-free translation screening to reveal new inhibitors with unexpected ribosome fates.

Poster 110

Single-molecule dissection of pathogenic AGO2 variants implicated in Lessel–Kreienkamp syndrome

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Pathogenic variants in human Argonaute-2 (AGO2) disrupt microRNA-guided gene regulation and underlie the recently described Lessel–Kreienkamp syndrome. The original biochemical and cellular analyses revealed that several disease-associated mutations alter AGO2 target-binding dynamics, guide selectivity, and targeting fidelity, with G733R displaying pronounced defects in RISC assembly and structural stability (Liu et al., 2025). Building on these findings, we are collaborating with the authors to further characterize the pathogenic AGO2 variants G332P and G733R using single-molecule Förster resonance energy transfer (smFRET). This approach enables real-time resolution of guide and target engagement, dissociation kinetics, conformational transitions, and Dicer-mediated interactions. By directly observing structural dynamics and RNA-binding behaviour at the single-molecule level, our study aims to define how these variants perturb AGO2's conformational landscape and catalytic cycle. The resulting mechanistic insights will refine the molecular basis of AGO2-associated neurodevelopmental pathology and help establish quantitative structure–function relationships for disease-linked Argonaute variants.

Poster 111

Function of m^{6}A mRNA modification during meiosis in yeast

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Most eukaryotic mRNAs transcribed by RNA polymerase II are modified post-transcriptionally with a 5' cap, a 3' poly(A) tail, and spliced introns. In addition, internal adenosines can be methylated to form N^6-methyladenosine (m^{6}A), a prevalent modification influencing gene expression, development, and disease. m^{6}A affects processes such as pre-mRNA splicing, translation, and mRNA decay, yet its precise molecular roles remain incompletely understood, partly because few functional sites have been validated.
In the yeast *Saccharomyces cerevisiae*, m^{6}A deposition occurs exclusively during meiosis through the MIS complex containing the methyltransferase Ime4 (ortholog of METTL3). The reader protein Pho92 (homolog of YTHDF1) recognizes m^{6}A via its YTH domain and mediates downstream effects on mRNA stability and translation (Scutenaire et al., 2022). m^{6}A-mediated regulation has been shown to control meiotic recombination, and even the loss of a single methylation site can delay meiosis.
The main goal of my PhD is to decipher how m^{6}A influences mRNA fate during meiosis. I focused on identifying Pho92's interactors dependant on the m^{6}A mark. Using a combination of genetic, biochemical, and high-throughput approaches, I identified a specific interaction between Pho92 and Not1, a CCR4-NOT subunit involved in mRNA deadenylation. I also investigated the consequences of this interaction on the methylated mRNA fate.

Scutenaire, J., Plassard, D., Matelot, M., Villa, T., Zumsteg, J., Libri, D., & Séraphin, B. (2022). The S. cerevisiae m6A-reader Pho92 promotes timely meiotic recombination by controlling key methylated transcripts.

Poster 112

NCCR RNA and disease bioinformatics platform

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The aim of the NCCR RNA and disease bioinformatics platform is to help member research groups get accurate and reproducible biological insights from their experiments by providing high quality and insightful bioinformatics and data analytics. The team is composed of Dr. Aleksei Mironov (University of Basel), Dr. Virginie Ricci (University of Lausanne) and Dr. Puneet Sharma (ETH Zürich). Our services range from consultation about study design and experimental set-up to bioinformatic analysis of public or newly generated data. Present staff members have expertise in RNA-seq, single cell RNA-seq, ribosome profiling, CLIP, ChIP-seq, ATAC-seq, Bisulfite-seq, CRISPR screens, whole genome and exome sequencing, third generation sequencing technologies (e.g., Oxford Nanopore, PacBio). These diverse types of data and analyses require a special focus on reproducible research which we support through best practices, version control and automation via workflows and containers. We provide customized solutions tailored towards the needs of the project and help our collaborators tell extraordinary stories.

Poster 113

Understanding the assembly and function of the 40S hnRNP ribonucleosome

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Heterogenous ribonucleoproteins (hnRNPs) are an important class of RNA binding proteins (RBPs), integral to several RNA-related processes. While prior research has focused on the individual function of these hnRNPs, a crucial and understudied aspect of pre-mRNA regulation is that hnRNPs, mainly of the hnRNP A and C families, form a higher order 40S ribonucleoprotein complex called the ribonucleosome. While the biochemical composition of this complex was defined in a previous study [1], its precise assembly, function, and regulatory mechanisms remain unknown. To elucidate the molecular mechanisms governing ribonucleosome assembly, we have generated a set of hnRNPA1 mutants and tested the ribonucelosome assembly in cells expressing these mutants while in parallel the endogenous hnRNPA1 is knocked down. We found that these mutants were interacting poorly with the hnRNPC protein and hence, compromised ribonucelosome assembly. We are currently investigating the functional consequences of this compromised ribonucleosome structure, specifically by analyzing altered pre-mRNA splicing and nuclear export of mRNA in cells harboring these assembly-defective hnRNPA1 mutants. In parallel, we are searching for ribonucleosome-specific post-translational modifications (PTMs) on hnRNP components to define the dynamic regulatory mechanisms governing complex stability and formation.

[1] Domanski et al. (2022) Nucleic Acids Res 50(11):6300-6312

Poster 114

Determining the RNA Interactome of Glycolytic Enzymes

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An intriguing example of non-canonical protein-RNA interactions is the association of several metabolic enzymes with diverse RNA species, including mRNAs. Recent observations support the notion that such RNA interactions can modulate (riboregulate) enzymatic activities; however, it remains unclear which RNA species are present in sufficiently large copy numbers to affect metabolic enzyme activity in cells.
Previous data from our lab suggested that small non-coding RNAs, including tRNA-derived fragments (tDRs), associate with various glycolytic enzymes. Most of these tDRs are stress-induced degradation products of tRNAs, some of which are linked to diverse biological processes and diseases.
This project seeks to test a hypothesis stating that stress-induced interactions of glycolytic enzymes with small non-coding RNAs re-program cellular metabolism during the stress recovery.
To define the RNA interactomes of selected glycolytic enzymes, both biased and non-biased approaches are being pursued. CLIP-seq and high-sensitivity capture of endogenous RNA-protein interactions will be performed before and after exposure of somatic cells to particular stressors. This will allow mapping of RNA interactions in situ, and defining candidate RNAs for functional assays.
In addition, candidate enzyme–tDR interactions have already been validated using radiolabeled electrophoretic mobility shift assays (EMSA). Furthermore, in vitro enzyme kinetics in the presence of defined small RNAs have been performed. Ongoing work aims to determine whether these interactions measurably alter glycolytic flux.

Poster 115

Integrating modified nucleotides into RNA secondary structure predictions

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RNA modifications can significantly impact RNA structure and function, yet traditional structure prediction methods often overlook these important molecular marks. Since version 2.6, the ViennaRNA package supports the inclusion of modified nucleotides in RNA secondary structure prediction. However, the lack of energy parameters for most modifications continues to limit the accuracy of computational predictions, even though it is well established that many modifications can influence RNA folding.
While physics-based models remain essential for RNA structure prediction, experimentally determining energy parameters for all possible modifications is not feasible. Computational approaches, particularly molecular dynamics (MD) simulations, offer a promising alternative. We use the Transformato tool to automate the setup of MD simulations, which simplifies the exploration of free energy differences between modified and unmodified nucleotides.
On the one hand, we demonstrate the structural impact of these modifications across different contexts, with a focus on tRNA due to data availability. On the other hand, we estimate Nearest Neighbor parameters from MD-derived free energies. These efforts aim to support more accurate and flexible RNA structure prediction within the ViennaRNA framework, without relying exclusively on experimental data.

Poster 116

Rational design of RNA-guided molecular tools for the precise integration of therapeutic DNAs

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The present repertoire of genome editing tools offers diversified and goal-oriented systems that are optimised for a specific editing application. These include the use of Cas9 nucleases for disruption of genes, base editors for reverting point mutations, and prime editors for RNA-templated short insertions. The targeted insertion of long DNA stretches in the genome of human cells, however, remains a challenging task as the integration rates remain low. On the other hand, transposase-mediated gene integration is highly efficient and offers a safe mechanism avoiding DNA breaks but suffers from very high off-target activity. Bacterial CRISPR associated transposases like Tn6677 overcome this by an RNA-guided DNA binding domain which enables the precise targeting of their transposons. Since such systems remain inefficient in eukaryotes, we use a recently reported group of tiny eukaryotic RNA-guided DNA binding proteins as the chassis for an engineering efforts to generate fusion proteins with the catalytically active domains of various transposases, integrases or reverse transcriptases. Through rational-design and structure-based predictions we aim to improve the performance of these tools. This includes optimising the target site recognition strength, and reducing off target binding due to unspecific DNA interactions by the catalytic domains. The performance of all fusion constructs are evaluated by qPCR by comparing the abundance of modified and unmodified DNA at the target location, as well as to the general abundance of the DNA cargo. Achieving safe, efficient, and precise method for the integration of genomic cargos will enable the clinical use of curative gene switches.

Poster 117

Investigating the Medical Potential of Alternative splicing in Cancer Therapies

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Cancer is a complex disease with manifold causes and manifestations. Finding effective treatments for cancer patients is challenging. Comprehensive genomic profiling assays are currently the gold standard in identifying molecularly guided targeted therapies. However, many cancer patients do not respond to the recommended drugs despite measuring the mutational profile comprehensively. Experimental and virtual drug screens could help Molecular Tumor Boards prioritize treatment suggestions. However, most predictors base their predictions solely on omics and gene expression data with limited success. Information on alternative splicing changes has so far been ignored despite the knowledge that alternative splicing is a hallmark of cancer and drug resistance development. Utilizing our expertise in software development for Molecular Tumor Boards and the analysis of alternative splicing changes in cancer, we are developing the first drug response and resistance prediction tool that uses interpretable machine learning algorithms and alternative splicing analysis. The project focuses on publicly available pharmacogenomic data on lung, colon, ovary, kidney, and pancreas cancers and predict FDA-approved cancer drugs. This study highlights the importance of alternative splicing in drug responses and provides clinicians with software tools to make better therapy decisions and help patients fight their detrimental diseases.

Poster 118

Engineering AI-guided LNPs for cell-specific mRNA delivery

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Lipid nanoparticles (LNPs) have emerged as a promising delivery system for various therapeutic payloads, including mRNA vaccines and in vivo gene editing therapies. However, a major challenge in LNP-based delivery is their inherent tendency to accumulate in the liver. To address this critical issue, we are focused on engineering LNPs with enhanced cell-specific targeting capabilities. While decoration of LNPs with antibodies has been used extensively, the generation of new antibodies or nanobodies is a complex, costly, and slow process. Recent developments in AI tools such as BindCraft now enable the design of de novo binders (DNBs) for a given target of interest. We are investigating the potential of such DNBs to guide LNP uptake via targeting a surface receptor or antigen, creating a new class of functionalised LNPs. The specificity of these targeted LNPs is assessed by delivering fluorescent mRNA to cell lines expressing the targeted receptor. For example, LNPs decorated with a HER2 tyrosine-kinase receptor-targeting DNB show a 35-fold greater preference for transfection of K562 cells expressing HER2 versus wild-type HER2 negative cells. Due to the flexibility of designing a DNB for almost any given target antigen, compared with the time-intensive nature of antibody development, DNB-LNPs may offer a more adaptable and rapid approach to non-hepatic targeting. By developing LNPs capable of targeted delivery to specific cell types or organs beyond the liver, this project aims to expand the therapeutic applications of mRNA and gene therapies.

[1] Pacesa, M., Nickel, L., Schellhaas, C. et al. One-shot design of functional protein binders with BindCraft. Nature 646, 483–492 (2025). https://doi.org/10.1038/s41586-025-09429-6

Poster 119

Beyond Bulk: How scRNAseq, 3' UTR length and alternative splicing contextualises and advances bulk transcriptomic analysis

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Transcriptomic analysis has allowed researchers to investigate how an individual's gene expression is modulated by *Mycobacterium tuberculosis* infection and antimycobacterial drugs. However, most studies have only analysed bulk gene expression. This type of data cannot ascertain how cell-type composition and cell-type specific responses impact global expression patterns, nor how alternative transcript usage and 3' UTR lengths potentially impact a gene's post-transcriptional function and localisation. Thus, we sought to explore these novel datasets and analyses using pre- and post-treatment clinical samples from Tanzanian patients.

We performed paired bulk and single cell RNA sequencing (scRNAseq) on peripheral blood mononuclear cells (PBMCs) from 24 Tanzanian patients immediately before and five months after treatment for pulmonary tuberculosis. Performing classic bulk transcriptomic analysis, we confirmed previously published gene signatures, notably the downregulation of immunoglobulin-associated genes after treatment (e.g. IGHA1-2, IGLV2-8). Through our scRNAseq analysis, we demonstrate that this global signature is primarily driven by changes in cell-type composition, not cell-type specific expression changes. We also identified a number of non-differentially expressed genes that change in 3' UTR lengths and splicing patterns during treatment, potentially highlighting additional genes that may play a role in pathogen clearance and patient recovery.

These findings highlight the value of scRNAseq and non-expression bulk analyses in recontextualising and building on global gene expression patterns. This facilitates a greater understanding into the complex biology of tuberculosis. Further analysis into applying deconvolutional and multiomics integration pipelines on publicly available bulk RNAseq datasets will help assess the generalisability of our results.

Poster 120

RBMX functional retrocopy safeguards brain development

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Proper development and functioning of the cerebral cortex depend on the coordinated production, migration and differentiation of neurons. Disruption of one or several of these cellular processes can lead to neurodevelopmental disorders. The *RBMX* gene is located on the X chromosome, encodes the RNA-binding protein RBMX (also known as hnRNP-G), which regulates pre-mRNA splicing. *RBMX* has a retrocopy on human chromosome 1, called *RBMXL1*, which encodes a protein highly identical to the RBMX protein. We have gathered evidence supporting a functional redundancy between *RBMX* and *RBMXL1* at two levels: 1) at the molecular level, by analyzing their binding partners and RNA targets and 2) at the cellular level in neuronal progenitors through in vivo analysis (in utero electroporation) in the murine developing cortex. Additionally, we and other groups have assembled a cohort of 11 male individuals from nine unrelated families harboring pathogenic *RBMX* variants associated with intellectual disability and microcephaly. Most of these variants, which are located in the last exon, result in the production of nearly identical truncated proteins. Through a combination of in vivo analyses in the developing mouse cortex and in vitro assays, we demonstrated that N-terminal variants act via gain-of-function, whereas C-terminal variants act via partial loss-of-function mechanisms. Interestingly the severity of the phenotype varies among individuals carrying the same variant. Ongoing investigations aim to elucidate how the phenotypic expression of *RBMX* variants is modulated in humans, with evidence suggesting that the redundant function of its retrocopy, *RBMXL1*, during brain development may modify disease expression.

Poster 121

Localized translation at the nuclear pore complex

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In eukaryotic cells, translation predominantly occurs in the cytosol or at the surface of the endoplasmic reticulum. However, accumulating evidence suggests that subsets of mRNAs undergo translation at alternative subcellular sites. For instance, several nuclear-encoded mitochondrial mRNAs are translated at the mitochondrial outer membrane, and certain peroxisomal proteins are synthesized at the peroxisome surface.
In this project, we investigate localized translation at the nuclear pore complex (NPC), aiming to identify the specific mRNAs translated in the vicinity of the NPC. To achieve this, we used the BirA–AviTag proximity labeling system to biotinylate Rpl16a/b ribosomal proteins located near the nucleoporin Nup82. Following RNase I digestion and monosome purification using streptavidin-coated beads, we performed ribosome-protected fragment sequencing (Ribo-seq) to identify mRNAs associated with these ribosomes.
Our data reveal that the enriched transcripts predominantly encode proteins that localize to or function within the nucleus. Notably, coverage across these mRNAs was reduced at the 5′ ends of coding sequences. Furthermore, most of the enriched proteins possess N-terminal nuclear localization signals (NLSs), consistent with active translation near the NPC.
We propose that an NLS-binding import factor may interact co-translationally with nascent polypeptides, facilitating their recruitment to the NPC during translation. To validate this model, we plan to perform microscopic colocalization analyses of NPC components and the corresponding mRNAs.

Poster 122

Characterization of new variants in untranslated regions in Diamond-Blackfan anemia syndrome

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Diamond Blackfan anemia syndrome (DBAS) is a rare congenital disease caused by haploinsufficiency of a ribsomal protein gene. Although more than 20 ribosomal protein genes have been linked to this pathology, 25% of the mutations remain unknown and the variable penetrance of this disease is poorly understood. Here, we studied a family with two affected children bearing variants in non-coding sequences of the RPS26 gene: a mutation of the poly-adenylation signal and a polymorphism in the 5'UTR. We found that the mutation of the polyadenylation signal affects the maturation of the RPS26 mRNA. However it does not strictly segregate with the disease and cannot fully explain the condition of the affected children. We then evaluated the possible contribution of the 5' UTR polymorphism, which is common in the population. We established by RT-qPCR that this polymorphism is associated with a significant decrease in the amount of RPS26 mRNA. Transcription is not altered, suggesting that the half-life of these RNAs is shorter. This decrease in the amount of RPS26 mRNA is associated with a delay in the maturation of the small ribosomal subunit observed by Northern blot. We conclude that this polymorphism could contribute to the RPS26 deficiency causing the disease in these patients in combination with the mutation of the poly-adenylation signal.

Poster 123

Translational control of cell response to histidine depletion in T-cell acute lymphoblastic leukemia

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Cancers often display unique metabolic dependencies that can be exploited therapeutically. We investigated histidine, a semi-essential amino acid, as a potential metabolic vulnerability in T-cell Acute Lymphoblastic Leukemia (T-ALL). In a T-ALL mouse model, dietary histidine restriction extended survival most significantly compared to all other single amino acid deprivations. To define the mechanism, we used integrated multi-omics (RNA sequencing, ribosome profiling, and proteomics). Ribosome profiling provided evidence that histidine scarcity triggers ribosome stalling at histidine codons throughout the transcriptome. This stalling, reflecting the scarcity of charged tRNAHis, leads to pronounced ribosome collisions and activates the GCN2-mediated Integrated Stress Response (ISR) pathway, leading to a global downregulation of protein synthesis. We confirmed specific activation of the eIF2α kinases, GCN2 and PERK, but not other ER stress sensors (ATF6 and IRE1-XBP1). We demonstrate that GCN2 activation in our model was governed by a translational-transcriptional disconnect for core ISR components. While RNA-seq showed ISR transcripts were downregulated, ribosome profiling and proteomics revealed a significant increase in their translational efficiency, highlighting the need for multi-omic integration. This demonstrates that translational control is the dominant regulatory layer, effectively overriding transcriptional repression. Together with ISR activation, we consistently observed a suppression of cholesterol biosynthesis regulated by the transcription factor SREBP2. Our findings highlight a novel vulnerability in T-ALL, providing high-resolution mechanistic insight into how this cancer coordinates cellular programs for survival during nutrient stress.

Poster 124

Decoding tRNA structural information from chemical probing data

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The way RNA molecules fold is tightly linked to their function, evolutionary history, and capacity to explore conformational space under selective constraints. Yet, despite this importance, a central question remains: Where are all the RNA structures?
We address this question using transfer RNAs (tRNAs), which are highly structured, conserved, and extensively modified. Although their canonical fold is well established, chemical-probing datasets often contain signals that deviate from this consensus. We investigate whether these deviations arise from alternative conformations or heterogeneous read populations.
We analysed mutational profiling (MaP) signatures from published DMS/SHAPE-MaP datasets and unpublished data. Reads were classified as informative or non-informative based on the presence of MaP-induced mutations. Informative reads were further sorted according to the compatibility of co-occurring mutations with expected unpaired nucleotides in the canonical tRNA structure, including known modifications. This strategy allowed us to recalculate reactivity profiles for each read class using ShapeMapper2 and incorporate them as soft-energy constraints in structural prediction with RNAfold.
Across datasets, approximately 87% of reads were informative. Most were incompatible with the canonical fold, although the proportion of compatible reads varied across tRNA isodecoder families. Reactivities derived from compatible reads consistently shifted predictions toward the canonical structure, whereas non-compatible reads revealed alternative conformations.
Our results show that resolving SHAPE/DMS-MaP reads into canonical and alternative structural classes is essential for distinguishing signal from noise. Read-level compatibility determines which evidence should contribute to a reactivity profile and directly influences the predicted structure, uncovering co-occurring tRNA conformations that remain hidden in standard analyses.

[1] Yamagami, R., Sieg, J. P., Assmann, S. M., & Bevilacqua, P. C. (2022). Genome-wide analysis of the in vivo tRNA structurome reveals RNA structural and modification dynamics under heat stress. Proceedings of the National Academy of Sciences of the United States of America, 119(25), e2201237119. https://doi.org/10.1073/pnas.2201237119
[2] Vilardo, E. et al., pers. comm.

Poster 125

Geometric deep learning for context-aware protein interactomes

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Understanding molecular function requires accurate modeling of interactomes within specific cellular contexts, yet experimental observations of context-dependent interactions remain sparse and difficult to generate at scale. Computational approaches are therefore essential for inferring missing interactions and uncovering principles shaping context-specific protein–protein interactions (PPIs).
Building on PINNACLE, the first geometric deep learning framework for context-aware protein representations, we present an expanded and more biologically grounded framework that integrates improved interactome construction, richer protein features, and a redesigned learning architecture, addressing PINNACLE's key limitations. We refine cell-specific interactomes by expanding cellular coverage using the Human Brain Cell Atlas, enabling analysis of diverse human neuronal contexts relevant to neurodegeneration. We further redefine cell-specific PPIs to include essential and housekeeping proteins rather than restricting to marginally expressed genes. In addition, we include protein sequence and surface embeddings from foundation models, enabling inductive generalization and capturing intrinsic molecular properties that shape interaction specificity. Subsequently, we introduce an updated learning strategy incorporating heterophilic graph neural networks, improved sampling methods, cell-specific learnable virtual nodes, streamlined hierarchical relationships, and a masked autoencoding objective strengthening contextual learning.
Together, these advances yield substantial gains in context-specific PPI prediction. Moreover, our contextualized representations also improve predictions of subcellular localization, disease associations and mutation effects, including inference of the cell types most implicated in each phenotype. Our framework provides a powerful foundation for context-specific interactome modeling, with future extensions integrating additional modalities such as RNA to reveal how protein and RNA localization jointly contribute to regulation and dysfunction in neurodegenerative disease.

[1] Li, Michelle M., et al. "Contextual AI models for single-cell protein biology." Nature Methods 21.8 (2024): 1546-1557.

Poster 126

RNA synthesis platform: Beyond specialized oligonucleotide service

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The RNA Synthesis Platform of the NCCR "RNA & Disease," located at ETH Zurich, Hönggerberg, specializes in providing unique, specialized chemical tools for RNA projects of NCCR members and external collaborators. The platform also develops novel methodologies for synthesizing long, heavily modified oligonucleotides. Equipped with state-of-the-art oligonucleotide synthesis instruments and advanced organic chemistry expertise, the platform incorporates functional groups into DNAs and RNAs site-specifically. Oligonucleotides are synthesized on solid support using the phosphoramidite method, enabling precise and scalable production. Modified nucleoside phosphoramidites and post-synthetic conjugation techniques allow for the incorporation of specialized functional groups into the final molecule. Our platform has established numerous collaborations, providing tailored oligonucleotide tools that have significantly advanced various NCCR research projects. By continually innovating, the platform aims to expand the range of oligonucleotide modifications available and support groundbreaking discoveries in RNA biology and RNA-related diseases.

Poster 127

Early response to warm temperature in arabidopsis

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Temperature impacts plant development and fitness. Warm temperatures (24–30 °C) promote growth and induce morphological changes thought to aid cooling and acclimation [1], whereas temperatures above 30 °C cause growth arrest and a shorter life cycle [2,3]. Although these physiological responses differ, the molecular mechanisms enabling plants to distinguish between warm and heat-stress temperatures remain poorly understood. Because RNA structure and stability are temperature-sensitive [4], we hypothesize that warm temperatures reshape mRNA stability and secondary structure, thereby selectively routing specific transcripts toward translation or toward decay/sequestration [5], in contrast to the global translational stalling triggered by heat stress [6,7]. Here we show that *Arabidopsis thaliana* employs rapid, selective mRNA stability changes as an early post-transcriptional response to warm temperature (28 °C). These changes arise within 10 minutes and precede transcriptional reprogramming. Whereas previous studies reported that heat stress globally destabilizes about 25% of transcripts [8], we find that warm temperature instead induces targeted effects, destabilizing 13% and stabilizing 8% of modeled transcripts. Destabilized mRNAs are enriched for defence functions, while stabilized transcripts predominantly encode growth-related processes. This selectivity appears linked to multiple features, including conserved RNA structural motifs across 29 Brassicaceae species, GC-content differences in untranslated regions, and temperature-dependent shifts in RNA-binding protein interactions. Moreover, the dcp5-1 mutant displays delayed warm-induced hyponasty, indicating that the mRNA-decay machinery,DCP5 in particular [9],contributes to early warm-temperature responses. Together, these findings connect mRNA features with decay-factor activity and whole-plant outcomes, providing new insight into how plants fine-tune gene expression under warm conditions.

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Poster 128

Beyond nuclear export: UPF1's N-terminus regulates UPF2 and UPF3B levels

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic, translation-dependent pathway that degrades mRNAs containing premature termination codons and also regulates the turnover of fully coding transcripts. UPF1, an RNA helicase, is the central effector of NMD, and together with UPF2 and UPF3B, it forms a conserved core complex essential for this process.
Although UPF1 is predominantly cytoplasmic at steady state, it shuttles between the nucleus and the cytoplasm. Using transient overexpression assays, we previously identified and characterized a CRM1-dependent nuclear export signal (NES) within the N-terminus of UPF1. However, CRISPR-mediated disruption of the endogenous NES (UPF1-LF: L103A/F105A) in HEK cells did not result in nuclear accumulation of UPF1. Instead, UPF1-LF expressing cells exhibited elevated levels of other core NMD factors, including UPF2 and UPF3B.
To gain mechanistic insight into this upregulation, we performed a biochemical characterization of UPF1-LF cells, alongside control cells carrying silent mutations and the parental HEK cell line. The results obtained so far will be presented, including analyses of RNA and protein stability, as well as protein–protein interactions. Together, these findings aim to shed light on how UPF1's N-terminus contributes to the regulation of UPF2 and UPF3B levels.

Poster 129

Interplay Between rRNA Expansion Segments and rancRNAs in Translation Regulation in *Saccharomyces cerevisiae*

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Regulation of protein biosynthesis is crucial for cellular fitness and has become increasingly important as organisms evolved. Eukaryotic ribosomes acquired extensions in ribosomal proteins and ribosomal RNA expansion segments (ESs) to meet these heightened demands. Although ESs do not catalyze peptide bond formation, they modulate protein synthesis by influencing ribosomal dynamics and recruiting trans-acting factors. Over the past decade, ribosome-associated non-coding RNAs (rancRNAs) have emerged as key regulators of translation during stress responses, targeting the ribosome to modulate protein synthesis.
Here, we investigate whether ESs and rancRNAs interact during stress adaptation by generating 12 strains lacking individual rRNA expansion segments and exposing them to different stresses. Small RNAs from polysome fractions were then deep-sequenced to identify ES-dependent rancRNAs. Deletion of ES12S, an expansion segment in the small ribosomal subunit, altered rancRNA expression specifically during hyperosmotic stress. Among these, an mRNA-derived small RNA was upregulated in ΔES12S and directly inhibited translation of Rgc2, a positive regulator of the Fps1 glycerol channel essential for osmoregulation. Consistent with impaired Fps1 regulation, the ΔES12S strain exhibits a glycerol-channel dysfunction (closed-channel) phenotype. These findings reveal a functional link between ESs and rancRNA-mediated translation control during stress adaptation.

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Poster 130

Poly(2-oxazoline) lipids as PEG alternatives: modulating LNP surface structure

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Lipid nanoparticles (LNPs) are widely used as delivery systems for mRNA therapeutics and played an important role in the development of COVID-19 vaccines. Polyethylene glycol (PEG)–lipids are typically incorporated on the LNP surface to form a steric coating that enables extended systemic circulation. However, concerns regarding PEG-associated immunogenicity and the need to tailor nanoparticles for diverse therapeutic applications have increased interest in alternative polymeric materials. Poly(2-oxazoline) (POx)-based lipids have recently gained attention as substitutes for PEG-lipids, as they can be synthetically tuned in hydrophilicity, chain length, and terminal functionality, offering improved biocompatibility, altered organ-selectivity profiles, and reduced immune responses.
In this work, we incorporate poly(2-methyl-2-oxazoline) (PMeOx) lipids into LNPs, taking advantage of their hydrophilicity, which closely resembles that of the “gold standard” PEG. To investigate how POx lipids influence LNP structure and performance, detailed physicochemical characterization was performed. Nuclear magnetic resonance (NMR) spectroscopy was used to probe lipid packing and the distribution of surface-exposed lipids within intact nanoparticles.
Our analysis revealed increased exposure of ionizable lipids on the particle surface for POx-containing LNPs compared with PEG-containing formulations, indicating a modified interfacial environment. These differences in surface composition can create distinct biological identities, influencing cellular interactions and ultimately the efficiency of mRNA delivery.

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Poster 131

Design and Synthesis of TDP-43 targeting RNA-PROTACs

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Proteolysis targeting chimeras (PROTACs) can exploit the endogenous ubiquitin-proteasome system to induce degradation of proteins [1]. RNA-PROTACs are oligonucleotide conjugates, designed to bind RNA-binding proteins and an ubiquitin E3 ligase which allows transfer of ubiquitin onto a surface lysine of the RNA-binding protein. The polyubiquitinated RNA-binding is recognized and degraded by the proteasome [2].
First-generation RNA-PROTACs targeted RNA-binding proteins with short, conserved RNA-binding sequences. These RNA-PROTACs, using modified RNA conjugated to E3-ligase recruiters, induced proteasome-dependent degradation of LIN28 and RBFOX1 [2]. The second-generation RNA-PROTACs are being developed to induce degradation of RNA-binding proteins implicated in neurodegenerative diseases.
Neurodegenerative diseases are frequently associated with the accumulation of aberrant proteins, such as tau protein in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), and huntingtin in Huntington's disease (HD). PROTACs degrading these aberrant protein aggregates have been investigated as new therapeutic strategies in AD, PD and HD [3]. Similarly, in amyotrophic lateral sclerosis (ALS) and frontal temporal dementia (FTD), the RNA-binding protein TDP-43 mislocalizes to the cytoplasm, where aggregates are formed. These aggregates, along with the depletion of TDP-43 in the nucleus, eventually lead to motor neuron degeneration [4].
Here we present our efforts in the rational design of a RNA-PROTAC against TDP-43. We designed RNA ligands based on NMR structures of TDP-43 in complex with RNA. The binding of TDP-43 to unmodified RNA or 2'-modified phosphorothioated RNA was characterized in vitro. We synthesized a library of modified RNA-ligands conjugated to different E3-ligase recruited and see degradation of TDP-43 in cell models.

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Poster 132

High-content drug screens identify novel regulators of mRNA localization

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Several natural transcription inhibitors have been utilized as chemotherapeutic agents, serving as a foundation for an expanding array of small molecules designed to target transcription in cancer. Our previous research demonstrated that transcription inhibitors significantly influence mRNA transcript localization and nuclear export. These shifts in localization provide inherent resistance to cellular catastrophe resulting from transient global transcription inhibition. Building on these findings, we developed and executed a high-content 384-well screen to assess whether we could better understand the mechanism of transcriptional induced RNA retention as well as whether other inhibitors may also have previously unknown effects on mRNA localization. This screen revealed that several clinically approved inhibitors as well as compounds derived from natural sources alter mRNA localization independently of their reported primary functions. We aim to leverage these findings to better understand the mechanisms of action of these drugs, as well as the biological processes underlying mRNA trafficking.

Poster 133

Codon-specific regulation of translation by polyamines independent of eIF5A hypusination

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Neuroblastoma is a highly lethal pediatric malignancy depending on local polyamine biosynthesis. Inhibition of polyamine biosynthesis via difluoromethylornithine (DFMO) was recently approved by the FDA for neuroblastoma treatment. Our work shows that the efficacy of DFMO can be significantly enhanced by dietary restriction of proline and arginine in the TH-MYCN neuroblastoma mouse model, triggering tumor differentiation and extending survival. Mechanistically, ribosome profiling reveals a unique translation defect characterized by ribosome stalling specifically at codons ending in adenosine (A). Such A-ending codons are selectively enriched in cell cycle genes but low in neuronal differentiation genes. Importantly, we show that this codon-specific stalling arises independently of post translational modification of eIF5A by spermidine, called hypusination. Using cellular models inducing genetic or pharmacological loss of the hypusine-modifying enzyme DHPS or pharmacological inhibition of MYCN does not share the A-ending codon stalling phenotype. Supplementation of polyamines fully rescues the growth defects caused by DHPS knockdown in a polyamine depletion background. This confirms that the translational impairment observed under polyamine depletion in neuroblastoma is independent of hypusination loss, predominantly conferring phenotypes in immune contexts. Together this work highlights a translation defect in neuroblastoma upon polyamine depletion that is independent of eIF5A hypusination. This highlights a context-specific role of polyamines and potential novel therapeutic strategies for targeting translational control in cancer and beyond.

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Poster 134

The function of non-coding RNAs in the neurophysiological profile of Williams-Beuren Syndrome

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William-Beuren Syndrome (WBS) is a complex neurodevelopmental disorder with polysystemic effects, including distinctive defects in the neurocognitive domain. Two transcription factors, General Transcription Factor 2I (GTF2I) and GTF2I repeat domain-containing 1 (GTF2I RD1), are strong candidates for mediating the observed neurocognitive phenotypes, based on results from animal and cellular models [1,2]. However, the molecular mechanism downstream of GTF2I/RD1 has not yet been described. Our lab identified several dysregulated miRNAs of the miR-379-410 cluster in WBS human iNeurons [3]. Therefore, we hypothesize that miRNAs, and possibly other non-coding RNAs, could be mechanistically linked to the phenotypic changes mediated by GTF2I/RD1. We engineered human induced Pluripotent Stem Cell (hiPSC) lines harbouring homozygous and heterozygous deletions of these genes, using CRISPR/Cas9 gene editing technology. The knockout cell lines were validated using genome editing efficiency methods, namely T7 endonuclease assay and Tracking Indels by Decomposition (TIDE) analysis, followed by assessment of mRNA and protein levels. The above cell lines show a delayed proliferation rate compared to control cell lines. Next, we aim to study the morphological and electrophysiological changes in GTF2I/RD1 iNeurons and manipulate the expression of candidate non-coding RNAs to reverse the phenotype. Overall, this project will give insights into the pathophysiology underlying the neurocognitive defects in WBS and potentially reveal an involvement of non-coding RNAs in disease development.

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Poster 135

Intersections of NMD and MDA5 in cellular dsRNA surveillance

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Previous work from our lab uncovered a type I interferon response in the livers of mice carrying an inactive version of the nonsense-mediated mRNA decay (NMD)-specific endonuclease SMG6 [1]. Using ex vivo fibroblasts, we further showed that this response is a cell-autonomous consequence of NMD inhibition and operates through the double-stranded RNA (dsRNA) sensor MDA5 (encoded by Ifih1). Although MDA5 is classically known to sense viral dsRNA, a recent study [2], demonstrated that it can also bind cellular dsRNA. These observations raise the question of whether MDA5 and NMD act on overlapping RNA substrates to maintain cellular homeostasis or to initiate early innate immune responses against faulty transcripts.
To address this, we introduced an ALFA-tag [3] into the endogenous Ifih1 locus to tag MDA5 in Smg6-mutant cells, allowing us to identify MDA5-associated NMD substrates through cross-linking and immunoprecipitation (CLIP). We will present new insights into the interplay between dsRNA sensing and NMD.

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Poster 136

TRIM71-Congenital Hydrocephalus: The Impact of RNA-binding Protein Dysfunction in Brain Development

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Congenital hydrocephalus (CH) affects approximately 1 in 1,000 newborns, making it one of the most common neurodevelopmental disorders. Traditionally, it has been perceived as a fluid regulation issue caused by either excessive productionor inefficient drainage of cerebrospinal fluid (CSF) in the brain ventricles. Recently, genetic studies using whole-exome sequencing identified the RNA-binding protein TRIM71 as a CH-risk gene. TRIM71 regulates stem cell fate by triggering degradation of mRNA targets, and the observed heterozygous mutations occurred in the RNA-binding domain of TRIM71. Investigation of analogous mutations in a mouse model revealed precocious neuroprogenitors differentiation, resulting in altered cortex development, secondarily leading to CH. Therefore, in a subset of patients, CH appears to be a neurodevelopmental disease and understanding the underlying mechanism may help for better diagnostic and treatment. Using mouse embryonic stems cells (mESCs), we find that TRIM71s form dimers which are essential for stable binding of mRNA target as well as recruitment of mRNA degradation cofactors. Interestingly, heterodimers of wild-type and mutant TRIM71 fail to interact with cofactors, and ectopic expression of TRIM71 mutant leads to target upregulation in a dimer-dependent manner and mislocalisation. Altogether, it suggests a dominant negative activity of the mutant which could explain TRIM71 CH-phenotype. We propose that this causes precocious upregulation of mRNA targets in neuroprogenitors cells resulting in premature differentiation, and we are now implementing brain organoid system to assess it.

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FAQ

Breakfast
Please have breakfast at your hotel, then join the symposium at the Concert Hall afterward.

Bars
Bar Murütsch at Hotel Laudinella, open daily till 3am. Vic's Bar at Reine Victoria, open daily till 11pm

Certificate of Attendance
Please contact nccr-rna-and-disease@unibe.ch after the symposium if you need a certificate of attendance.

Check In
Please proceed directly to your hotel reception for individual check-in. You will receive your room details and key there.

Check Out
Please check-out by 10 am on your departure day, by leaving your room empty and return the key at the reception. Luggage storage is available at each hotel or in front of the concert hall for those staying at the Youth Hostel.

Contact / Support
For hotel-related matters, please contact your hotel reception directly. For symposium-related matters, please come by the Symposium desk during the coffee breaks or contact us by e-mail (see contact information below).

Dietary Restrictions
Please inform the respective restaurant directly about any dietary requirements.

Dinner
Dinner is served buffet-style at the Stüva Restaurant, except on Wednesday evening. On Wednesday, dinner is served at Hotel Reine Victoria after the Poster Session (see program for details).

Drinks
A limited number of drinks are covered by your voucher, which you receive during registration Drinks during the Flying Dinner are covered too. Additional drinks are at your own expense. Drinking water is available all day.

Getting Around
St. Moritz Bad is very walkable, and the venues are located close to each other. The Youth Hostel is the furthest away, approximately a 12-minute walk. Keep in mind to dress warm.

Hotel Information
Your assigned hotel was communicated by email. A participant list is also available at the Symposium Desk.

Key / Room information
Please collect your room key at your hotel reception.

Lunch
Lunch is served buffet-style at the Stüva Restaurant.

Parking
There are a limited number of parking spaces available (CHF 15 per night). Please contact the hotel directly for reservations.

Power Outlets / Electrical Plugs
Switzerland uses Type J power outlets (Swiss standard) with a voltage of 230 V. Plugs from many other countries (e.g. EU, UK, US) may not fit directly. An adapter may therefore be required. Please ensure you bring a suitable travel adapter for your electronic devices.

Program
Please refer to the booklet for details and check the online program regularly for updates.

Public Transport
Public transport is at your own expense. Tickets can be purchased at vending machines at bus stops or online at www.sbb.ch/en.

Registration
Registration takes place at Hotel Laudinella prior to the first session on Monday (Symposium Desk).

Venue
Symposium venues are Hotel Laudinella and Hotel Reine Victoria. Please refer to the program overview for session details.

Voucher Drinks
You will receive a drinks voucher during registration. The voucher is valid for four glasses of soft drink, wine, beer, or prosecco and can be redeemed at the Lobby Bar of Hotel Laudinella or Hotel Reine Victoria.

Water
Tap water is of high quality and safe to drink without a filter.

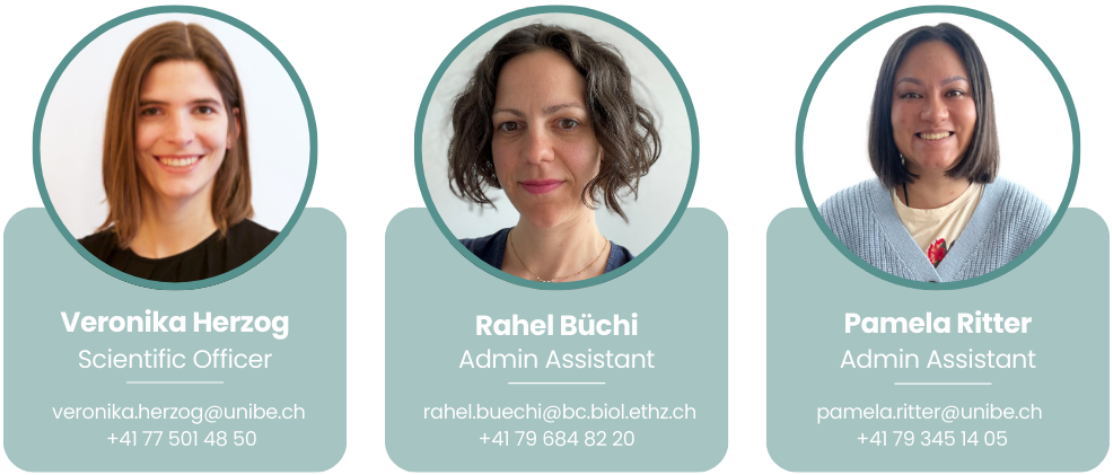
Weather
January in St. Moritz is typically cold and wintry, with snow and sub-zero temperatures. Please dress warmly and wear appropriate winter footwear.

WiFi
WiFi is available at all three hotels. Please contact the hotel reception for access details.

CONTACT INFORMATION

If you need any assistance during the event, please don't hesitate to reach out to us. You're welcome to stop by the Symposium Desk in front of the Concert Hall at Hotel Laudinella or contact us by email.

We'll be happy to help!



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