13th Structure Integration Function & Reactivity of RNA

18 - 20 November 2024 - LYON

Amphithéâtre Mérieux
Ecole Normale Supérieure de Lyon

Dear colleagues,

Welcome to Lyon for the 13th SifrARN conference!

This meeting, organized under the auspices of the French Society of Biochemistry and Molecular Biology (SFBBM), is being held in Lyon for the first time. With nearly 300 participants joining from both academic laboratories and industry, the 13th SifrARN reflects the great dynamism of our community. Together with the Scientific Committee, we have put together an exciting program that covers most aspects of the RNA field. There will be a special focus on the numerous connections between RNA and diseases, as well as the use of RNA molecules as clinical tools.

We are delighted to welcome three distinguished keynote speakers, Roland Beckmann, Maria Carmo-Fonseca (FEBS Lecture) and Matthias Hentze. The Scientific Committee faced the challenging task of selecting 49 oral presentations from the 82 submitted abstracts, and we will have a total of over 100 poster presentations.

We would like to thank our institutional partners and sponsors for their generous support.

Above all, this event would not be possible without your participation, so thank you for joining us. We hope you enjoy your stay in Lyon and wish you a vibrant and engaging meeting!

Cyril Bourgeois on behalf of the Organizing Committee.

Organizing Committee

Cyril Bourgeois, LBMC, Lyon ; **Didier Auboeuf**, LBMC, Lyon ; **Frédéric Catez**, CRCL, Lyon ; **Francesca Fiorini**, MMSB, Lyon ; **Virginie Marcel**, CRCL, Lyon : **Sylvie Mazoyer**, CRNL, Bron ; **Vincent Mocquet**, LBMC, Lyon ; **Franck Mortreux**, LBMC, Lyon ; **Emiliano Ricci**, LBMC, Lyon ; **Ariane Pachins**, SFBBM

Scientific Committee

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Florence Besse, IBV, Nice
Sébastien Campagne, ARNA, Bordeaux
Sébastien Durand, CRCL, Lyon
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Martine Simonelig, IGH, Montpellier
Carine Tisné, IBPC, Paris

Day 1 | Monday 18 November

11:00 – 13:00 Welcome and registration

13:00 – 13:15 INTRODUCTION
Cyril Bourgeois

13:15 – 14:15 KEYNOTE LECTURE
Matthias Hentze EMBL, Heidelberg, Germany
"Riboregulation: a new RNA function offering therapeutic opportunities"

14:15 – 15:05 SESSION 1: RNA MATURATION
Chairs: Carine Tisné & Martin Dutertre

14:15 – 14:30 **Serhii Pankivskyi** SABNP, Evry, France — O1
"Proximity labeling reveals the complexity of RS domain-dependent interactions of U2AF2 for alternative splicing"

14:30 – 14:45 **Cyril Jovani** Lyon Neuroscience Research Center, France — O2
"Role of the minor spliceosome component U4atac snRNA in gene expression during zebrafish development"

14:45 – 15:00 **Wojtek Galej** EMBL Grenoble, France — O3
"Structural basis of the U12-dependent intron recognition"

15:00 – 15:05 **Gwendal Dujardin** University of Oxford, UK — O4-F
"Transcription and mRNA processing recovery after global Pol II promoter proximal blocking"

15:05 – 15:30 COFFEE BREAK

SPONSOR PRESENTATION BY BGI
Ruoyang ZHAI

15:30 – 16:40 SESSION 1-bis: EPITRANSCRIPTOME
Chair: Iouri Motorine

15:30 – 15:45 **Christine Allmang** IBMC, Strasbourg, France — O5
"Repertoire of mRNAs with trimethylated caps in mammals and study of their translation"

15:45 – 16:00 **Alexandre David** Institut de recherche en Cancérologie de Montpellier, France — O6
"Two microbiome metabolites competing for tRNA modification impact mammalian cell proliferation and translation quality control"

16:00 – 16:15	**Damien Bregeon** Sorbonne Universités, Paris, France “Exploring a Novel Class of Flavoenzymes: Identification and Biochemical Characterization of Ribosomal RNA Dihydrouridine Synthase.”	O7
16:15 – 16:30	**Louna Fruchard** Institut Pasteur, Paris, France “The Dihydrouridine synthase DusB : a role in bacterial adaptation to oxidative stress”	O8
16:30 – 16:35	**Pierre Klein** The Francis Crick Institute, London, UK “m6a methylation orchestrates IMP1 regulation of microtubules during human neuronal differentiation”	O9-F
16:35 – 16:40	**Sandra Blanchet** Institut de Biologie Intégrative de la Cellule, Gif-Sur-Yvette, France “The impact of RNA modifications on the translation fidelity”	O10-F

16:40 – 17:00 COFFEE BREAK

17:00 – 19:00	SESSION 2: DIAGNOSTIC, VACCINAL & THERAPEUTIC RNA Chairs: *Marie-Dominique Galibert & Chantal Pichon*	
17:00 – 17:15	**Léa Cerato** Institute for Advanced Biosciences, Grenoble, France “Circular RNAs: new players in the dialogue between chemotherapy-resistant lung cancer cells and dendritic cells?”	O11
17:15 – 17:30	**Frédéric Ducongé** MIRCen, Fontenay aux roses, France “Aptamer binding footprints (AptaFOOT-Seq) to improve the diagnosis of synucleinopathies.”	O12
17:30 – 17:45	**Sebastien Campagne** ARNA, Bordeaux, France “Rational Design of RNA-Based Therapeutics for Cancer Treatment.”	O13
17:45 – 18:00	**Erica Cirri** TEBUBIO, France “Unlocking the Future of RNA based vaccines: Tebubio's production solutions”	O14
18:00 – 18:15	**Delphine Allouche** SANOFI, France “Overview of Sanofi's mRNA Center of Excellence (CoE) and example of characterization for new mRNA vaccines: RNA secondary structure prediction at different mRNA vaccine stages”	O15
18:20 – 19:00	**Q & A and Discussion**	

19:00 – 21:30 DINER AND POSTER SESSION I

Day 2 | Tuesday 19 November

8:45 – 9:30	SESSION 3: RNA IN NON-INFECTIOUS DISEASES *Chairs: Sébastien Campagne & Fabrice Lejeune*	
8:45 – 9:00	**Pierre Barraud** Institut de Biologie Physico-Chimique, Paris, France "Dimerization of ADAR1 modulates site-specificity of RNA editing"	O16
9:00 – 9:15	**Martin Dutertre** Institut Curie, Orsay,France "Microprotein-coding intronic polyadenylation isoforms: A new genetic paradigm"	O17
9:15 – 9:30	**Paulo Da Costa** Institut de biologie moléculaire et cellulaire, Strasbourg, France "Tau mutations and short 5'UTR tau mRNAs boost tau translation"	O18

9:30 – 10:30	FEBS LECTURE **Maria Carmo-Fonseca** Instituto de Medicina Molecular João Lobo Antunes, Lisbon, Portugal "Promoter-proximal convergent antisense transcription"	

10:30 – 11:00	COFFEE BREAK SPONSOR PRESENTATION BY NEB *Morgane MOREAU*	

11:00 – 11:30	SESSION 3: RNA IN NON-INFECTIOUS DISEASES (continued)	
11:00 – 11:15	**Albertas Navickas** Institut Curie, Orsay, France "ZZEF1 drives a metastasis-suppressing tRNA expression program in breast cancer"	O19
11:15 – 11:30	**Laetitia Meulemans** Normandy Centre for Genomic and Personalized Medicine, Rouen, France "From the last intron to the 3'UTR: a high number of variants in/near MSH2 terminal exon alter RNA splicing of this tumor suppressor gene"	O20

11:30 – 13:00	SESSION 4: RNA TRANSPORT AND DEGRADATION *Chairs: Florence Besse & Hervé Le Hir*	
11:30 – 11:45	**Etienne Dubiez** EMBL Grenoble, France "Structural studies of nuclear cap-binding complexes shed light on fate determination mechanism of RNA Pol II transcripts."	O21
11:45 – 12:00	**Lucie Labeauvie** Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France "Multi-omics and genetics analyses of pat1-deficient yeast cells uncover a transcriptome-wide impact on poly(A)-tail length suppressed by a ribosomal protein mutation"	O22

12:00 – 12:15	**Toni Gouhier** Institut de biologie de l'ENS Paris, France “An unexpected role of the NMD machinery in targeting oligoadenylated mRNAs for degradation”	O23
12:15 – 12:30	**Adham Safieddine** Institut de biologie de l'ENS Paris, France “Cell cycle-dependent mRNA localization in P-bodies”	O24
12:30 – 12:45	**Sandra Duharcourt** Institut Jacques Monod, Paris, France “Control of small RNA dynamics during programmed DNA elimination in Paramecium”	O25
12:45 – 13:00	**Alexandre D'halluin** Institut de biologie physico-chimique, Paris, France “Embedding a ribonuclease in the spore crust couples gene expression to spore development in Bacillus subtilis”	O26
13:00 – 15:20	LUNCH AND POSTER SESSION II	
15:20 – 16:50	SESSION 5: CONTROL AND MECHANISM OF TRANSLATION *Chairs: Stefania Millevoi & Sébastien Durand*	
15:20 – 15:35	**Pauline Herviou** CRUK Scotland Institute, Glasgow, UK “The CCR4-NOT complex and codon optimality promote co-translational mRNA targeting to the ER.”	O27
15:35 – 15:50	**Emiliano Ricci** ENS de Lyon, France “Quantitative mass spectrometry of affinity-purified ribosomes uncovers ribosome interactome specialization during viral infections”	O28
15:50 – 16:05	**Ali Haidar** Institut de génétique humaine, Montpellier, France “Spatial organization of translation and translational repression in two phases of germ granules”	O29
16:05 – 16:20	**Salloum Soha** Institut de génétique humaine, France “Co-translational sorting enables a single mRNA to generate distinct polysomes with different localizations and protein fates”	O30
16:20 – 16:35	**Jonathan Jagodnik** Institut de biologie physico-chimique, Paris, France “30S-RiboSeq redefines the bacterial Ribosome Binding Site”	O31
16:35 – 16:50	**Victoria Nicolini** Institut de Recherche sur le Cancer et le Vieillissement, Nice, France “Glucocorticoid receptor activation improves dichotomy between translated and untranslated mRNA through active RNA storage in Processing-bodies”	O32
16:50 – 17:20	COFFEE BREAK SPONSOR PRESENTATION BY MICROSYNTH *Emeraude HADJ-ATTOU*	

17:20 – 18:50	SESSION 6: NON-CODING RNAS *Chairs: Karina Jouravleva & Martine Simonelig*	
17:20 – 17:35	**Patrice Vitali** Centre de Biologie Intégrative (CBI), Toulouse, France "The C19MC Gene Locus: Evolutionary Significance and Regulatory Functions in Placenta-Derived Cells."	O33
17:35 – 17:50	**Francesco Angelelli** Centre méditerranéen de médecine moléculaire, Nice, France "Comprehensive miR-21-binding site identification and mediated repression in hepatocytes"	O34
17:50 – 18:05	**Louise Velut** BioSanté IRIG, Grenoble, France "Analysis of microRNA and mRNA co-sequencing data at the single-cell level"	O35
18:05 – 18:20	**Maxime Wery** Institut Curie, Paris, France "Cryptic cytoplasmic long noncoding RNAs are pervasively translated in yeast and human cells"	O36
18:20 – 18:35	**Julien Ladet** ENS de Lyon, France "HBZ-Related Dysregulation in Circular RNA Biogenesis during Adult T-Cell Leukemia Development"	O37
18:35 – 18:50	**Quentin Dubois** INSA de Lyon, France "A natural single nucleotide mutation in the small regulatory RNA ArcZ of the Plant pathogenic bacteria Dickeya solani switches off its antimicrobial activities against yeast and bacteria, and impact its virulence."	O38

Please make sure that all posters are taken down by 6:00pm – Thank you!

19:30 – 23:00 GALA DINER AT THE PALAIS DE LA BOURSE

WITH

Day 3 | Wednesday 20 November

8:45 – 9:45	SESSION 7: BIOGENESIS AND STRUCTURE OF RIBOSOMES *Chairs: Reynald Gillet & Anthony Henras*	
8:45 – 9:00	**Christophe Dez** Centre de Biologie Intégrative (CBI), Toulouse, France “Ribosomal RNA synthesis by RNA polymerase I is regulated by premature termination of transcription”	O39
9:00 – 9:15	**Magali Blaud** Cibles Thérapeutiques et conception de médicaments, Paris, France “RNase W, a conserved ribonuclease family with a novel active site”	O40
9:15 – 9:30	**Gleizes Pierre-Emmanuel** Centre de Biologie Intégrative (CBI), France “Cytoplasmic uridylation monitors maturation of the 18S rRNA 3' end”	O41
9:30 – 9:45	**Schmitt Emmanuelle** Laboratoire de Biologie Structurale de la Cellule, France “Cryo-EM Structures of Saccharolobus solfataricus Initiation Complexes"	O42

9:45 – 10:45	KEYNOTE LECTURE **Roland Beckmann** University of Munich LMU, Germany "The Beginning and the End of the 40S Ribosomal Subunit"	

10:45 – 11:15	COFFEE BREAK	

11:15 – 11:45	SESSION 7: BIOGENESIS AND STRUCTURE OF RIBOSOMES (Cont.)	
11:15 – 11:30	**Anaïs Astier** Centre de Biologie Intégrative (CBI), Toulouse, France “A comprehensive, multi-omics and structure-function study of mutant 'onco-ribosomes' involved in Chronic Lymphocytic Leukemia.”	O43
11:30 – 11:45	**Virginie Marcel** Cancer Research Center of Lyon, France “Intricate ribosome composition and translational reprogramming in epithelial-mesenchymal transition”	O44

11:45 – 13:00	SESSION 8: RNA IN HOST-PATHOGENS INTERACTIONS *Chairs: Yoann Augagneur & Magali Frugier*	
11:45 – 12:00	**Makram Mghezzi-Habellah** ENS de Lyon, France "Hijacking of the RNA helicase, UPF1 reveals its proviral function during HTLV-1 infection."	O45
12:00 – 12:15	**Sara Moutacharrif** INSA de Lyon, France "Identification of a bifunctional RNA involved in the virulence of Dickeya dadantii"	O46
12:15 – 12:30	**Grégoire De Bisschop** Institut de Recherches Cliniques de Montréal, Canada "Deciphering the impact of SARS-CoV-2 infection on cellular RNA machineries localization and function"	O47
12:30 – 12:45	**Eva Kowalinski** EMBL Grenoble, France "SL RNA recognition by the unusual trypanosomatid cap-binding complex."	O48
12:45 – 13:00	**Sébastien Pfeffer** Architecture et Réactivité de l'ARN, Strasbourg, France "Modulation of antiviral innate immunity by the human Dicer protein"	O49

13:00 – 13:15 POSTER AWARDS & CONCLUSION
Chairs: Scientific committee & Organizing committee

13:15 – 14:00 LUNCH BOX PICK-UP
Available only for those who reserved at registration.

14:00 – 16:00 GUIDED TOUR OF "BIENNALE D'ART CONTEMPORAIN"
Available only for those who reserved at registration.

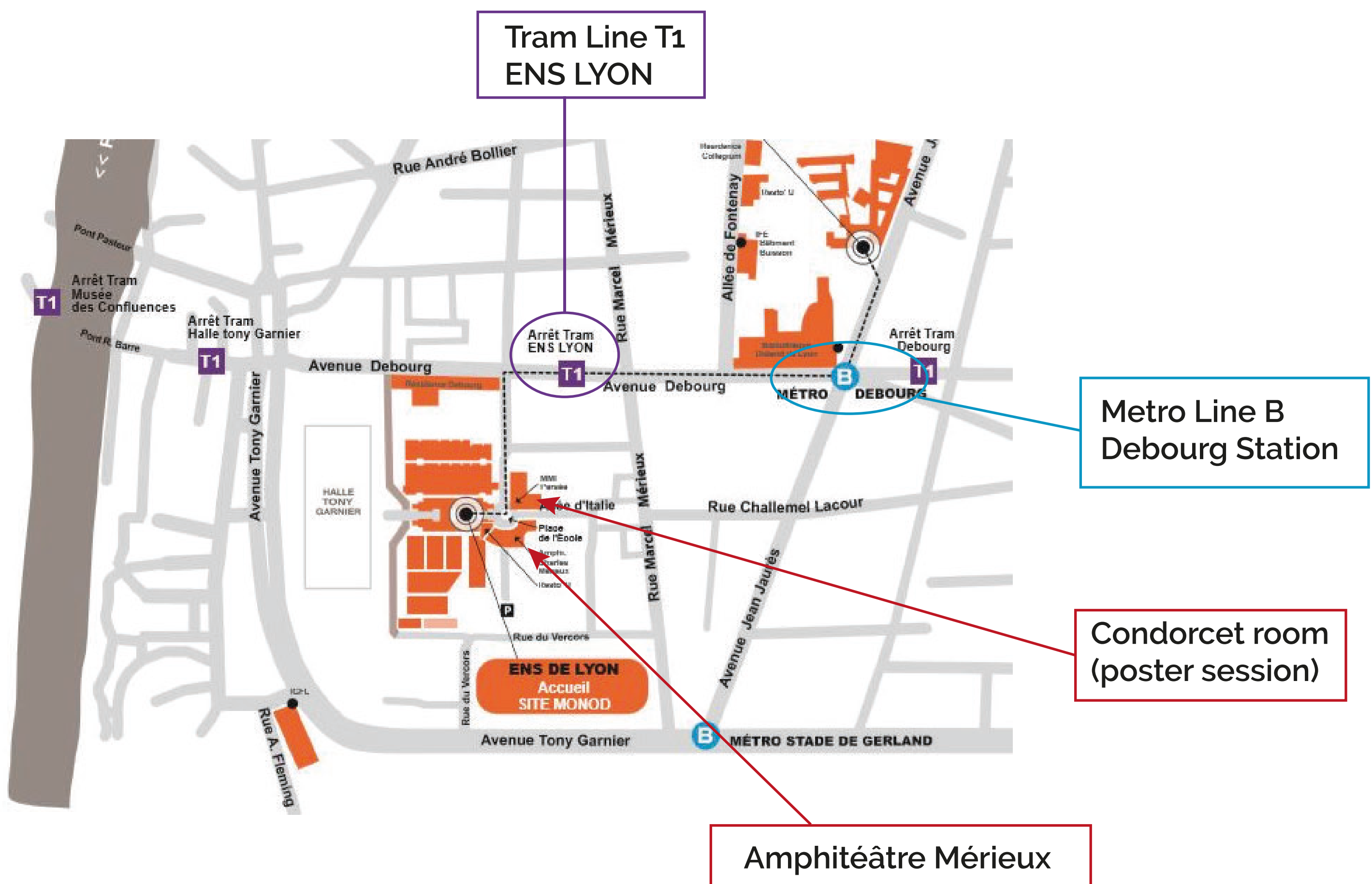
VENUE

The meeting takes place in the **Amphithéâtre Mérieux of the Ecole Normale Supérieure de Lyon**.
Address: 46, allée d'Italie, 69007 LYON
Google code PRHH+R7 Lyon
GPS: 45.729793148626946, 4.828234458201539

From Part-Dieu railway station, take metro line B, towards Oullins. Get off at "Debourg" station.

From Saint-Exupery Airport, there are several possibilities.
The easiest option is to take the Rhône Express. A dedicated ticket is required which can be purchased on their website: https://www.rhonexpress.fr ; or onsite.
Other options are available on the airtport's official website:
https://www.lyonaeroports.com/en/access-transports/travelling-lyon-airport-city-centre

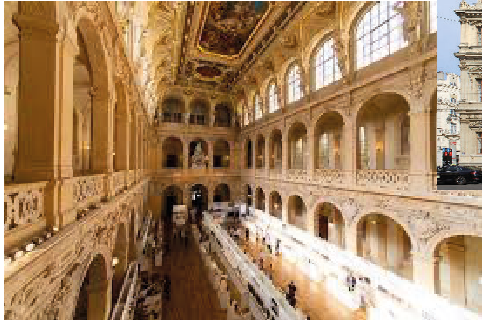
The public transportation company in Lyon is TCL (www.tcl.fr)
Tickets can be purchased before boarding, at Metro or Tram station.
It is possible to acces transportation directly with a credit card or any contact-less payment device. 48h and 72h tickets are also available but may not be profitable for this meeting.



Gala Diner

We are happy to welcome you at the **Palais de La Bourse** for the gala diner of the 13th Sifr ARN!

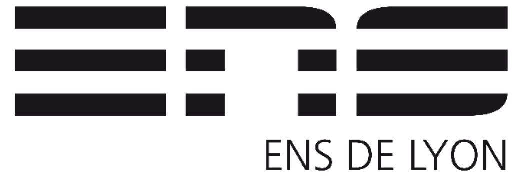
The gala evening will start at 7:30pm.
There is no specific dress code.

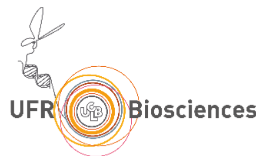


Diner provided by

Location
Place de la bourse, 69002 LYON
The accessible entry for individuals with limited mobility is at 21 Rue de la République.
Directions
From the congress site, take the metro at Debourg station on Line B towards "Charpennes". Transfer at Saxe-Gambetta to Line D towards "Gare de Vaise". At Bellecour station, transfer once more to Line A towards "Vaulx-en-Velin La Soie" and get off at Cordeliers station.

Institutional Support





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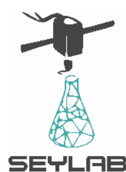

KEYNOTES

Riboregulation: a new RNA function offering therapeutic opportunities

Matthias W. Hentze

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EMBL, Heidelberg, Germany

Following the discovery that the RNA-binding proteome is far larger than previously anticipated (Castello et al., 2012; Hentze et al., 2018), riboregulation, the direct control of protein function by RNA, has begun to emerge as a new paradigm of biological control (Horos et al., 2019; Huppertz et al., 2022; Chatterjee et al., 2024). We are beginning to understand molecular mechanisms of riboregulation, and I will discuss their implications for cell biology, metabolism and disease mechanisms as well as the new therapeutic opportunities.

Castello, A., B. Fischer, K. Schuschke, R. Horos, B.M. Beckmann, C. Strein, N.E. Davey, D.T. Humphreys, T. Preiss, L.M. Steinmetz, J. Krijgsveld and M.W. Hentze. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell 149, 1393-1406, 2012.

Hentze, M.W., A. Castello, T. Schwarzl and T. Preiss, A brave new world of RNA-binding proteins. Nature Rev. Mol. Cell Biol. 19, 327-341, 2018.

Horos, R., M. Büscher, R. Kleinendorst, A.-M. Alleaume, A.K. Tarafder, T. Schwarzl, D. Dziuba, C. Tischer, E.M. Zielonka, A. Adak, A. Castello, W. Huber, C. Sachse and M.W. Hentze, The small non-coding vault RNA1-1 acts as a riboregulator of autophagy. Cell 176, 1054-1067, 2019.

Huppertz, I., J.I. Perez-Perri, P. Mantas, T. Sekaran, T. Schwarzl, F. Russo, D. Ferring-Appel, L. Dimitrova-Paternoga, E. Kafkia, J. Hennig, P.A. Neveu, K. Patil and M.W. Hentze. Riboregulation of Enolase 1 Activity Controls Glycolysis and Embryonic Stem Cell Differentiation. Mol. Cell 82, 2666-2680, 2022.

Chatterjee, A., M. Noble, T. Sekaran, V. Ravi, D. Ferring-Appel, T. Schwarzl, R. Rampelt and M.W. Hentze. RNA promotes mitochondrial import of F1-ATP synthase subunit alpha (ATP5A1). doi: https://doi.org/10.1101/2024.08.19.608659.

Promoter-proximal convergent antisense transcription

Maria Carmo-Fonseca

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Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Avenida Professor Egas Moniz, Lisboa, Portugal.

From yeast to humans, promoters of protein-coding (pc) genes serve as origins for widespread antisense non-coding RNA transcription. Most of the identified promoter-associated antisense RNAs correspond to transcripts divergent from the pc gene. However, there is another class of convergently transcribed antisense RNAs that often initiate within the first intron of the pc gene and overlap the promoter region. We introduced the term "promoter-proximal convergent antisense transcripts" (PCATs) to specifically describe these RNAs. We sequenced Pol II-associated nascent transcripts in human cells and developed a Bayesian statistical model for the de novo identification of promoter-proximal RNAs. Our analysis revealed that approximately 40% of pc genes are associated with PCATs, and these genes exhibit higher transcriptional activity compared to those without PCATs. Notably, PCATs were prevalent among genes transitioning from a transcriptionally inactive to an active state. Based on these findings, we propose that PCATs play a role in transcriptional activation.

FEBS Lecture

The Beginning and the End of the 40S Ribosomal Subunit

Roland Beckmann

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Gene Center, Department of Biochemistry, University of Munich LMU, Munich, Germany

The correct synthesis, modification, and maturation of eukaryotic ribosomal RNA is guided by numerous snoRNPs and assembly factors. By cryo-EM we observed that the essential H/ACA snoRNP, snR30, acts outside of the core of the 90S pre-ribosome to form a "satellite particle". This satellite particle comprises the snoRNP and the 18S rRNA platform subdomain, guiding its independent folding through localized structural interactions. Once properly assembled, the subdomain can be integrated into the core 90S pre-ribosome, facilitating further maturation. In general, ribosome abundance requires stringent regulation, particularly under stress. We found that in response to amino acid deprivation, atypical protein kinase RIOK3 recognizes and mediates the degradation of ubiquitylated 40S ribosomal subunits. Here, the E3 ligase RNF10 first ubiquitylates ribosomal proteins uS3 and uS5, leading to selective depletion of 40S subunits. Cryo-EM structures reveal that RIOK3 directly interacts with these ubiquitylated proteins, thereby promoting degradation and maintaining ribosome homeostasis. Together, these findings emphasize the importance of snoRNPs in ribosome assembly beyond their modification function and the surprising molecular mechanisms of selective targeting of 40S ribosomal subunits for degradation in response to cellular stress.

ABSTRACTS OF ORAL COMMUNICATIONS

- Oral communications O1 to O49

Proximity labeling reveals the complexity of RS domain-dependent interactions of U2AF2 for alternative splicing

***Pankivskyi Serhii* [1],** Kobayashi Asaki [1], Salone Jean De Matha [1], Gargoly Kimberley [1], Pastre David [1], Maucuer Alexandre [1]

1 - INSERM U1204/SABNP (France)

Splicing factor U2AF2 plays a pivotal role in 3' splice site recognition at an early step of spliceosome assembly. This process relies on numerous protein and RNA contacts mediated by folded domains. In contrast, multivalent interactions provided by low-complexity regions of splicing factors are experimentally less documented. These dynamic interactions are known to induce liquid-liquid phase separation and mediate the assembly of membraneless organelles. Using biochemical assays, proximity labeling, splicing reporter, and RNA-seq analysis we reveal the fundamental role of the low complexity RS domain of U2AF2 in alternative splicing. First, we observed in coprecipitation experiments that deletion of the RS domain compromised the interaction between U2AF2 and SF3B1 or RNA polymerase II. Mutagenesis of the RS domain revealed that the strength of the binding of U2AF2 to SF3B1 depends on the length and arginine content of the RS domain. To explore in depth the function of the RS domain, we designed a proximity labeling experiment to capture interactions of full-length or RS-deleted U2AF2 in living cells. This reveals a large network of RS domains dependent U2AF2 partners enriched in speckle proteins and RNA-binding proteins involved in several steps of RNA processing. Immunoprecipitation experiments confirmed that the RS domain of U2AF2 mediates interactions with a variety of other RS domains probably via multivalent interactions. We then document that the RS domain of U2AF2 is phosphorylated in cells. This modification improves U2AF2 solubility probably through reduced homotypic self-interactions. It also affects its interaction with partners. Hypophosphorylated U2AF2 mutant with S-to-A substitutions shows a significantly better binding to SF3B1 compared to wild-type and phosphomimetic S-to-D mutant. To decipher the importance of the RS domain for splicing, we first used a bichromatic splicing reporter. Removal of the RS domain of U2AF2 increased skipping of the alternative exon. Re-expression of the phosphomimetic mutant restored exon inclusion, in contrast to the hypophosphorylated mutant. Then, using RNA-seq, we observed that the removal of the RS domain of U2AF2 and its knockdown reduced cassette exon inclusion genome-wide. Cassette exons were differently sensitive to both perturbations of U2AF2 depending on the 3' splice site features. Moreover, exons flanked by shorter introns and located closer to speckles were more sensitive to RS domain deletion or U2AF2 depletion. Overall, we demonstrate that the low complexity RS domain of splicing factor U2AF2 mediates multiple interactions with its partners that often contain RS domains whereas phosphorylation of serine residues in the RS domain modulates these interactions. Finally, this low complexity domain recruits U2AF2 to speckles to assure the inclusion of alternative exons in regions where the concentration of splicing factors might be limiting.

Role of the minor spliceosome component U4atac snRNA in gene expression during zebrafish development

Jovani Cyril [1], Rabec Alexia [1], Khatri Deepak [1], Cologne Audric [1], Gaubert Marianne [1], Guguin Justine [1], Besson Alicia [1], Mazoyer Sylvie [1], Delous Marion [1]

1 - Centre de recherche en neurosciences de Lyon - Lyon Neuroscience Research Center (France)

Splicing is a crucial RNA maturation process that involves two machineries: the well- known major spliceosome, responsible for the splicing of > 200,000 major/U2 introns, and theminorspliceosome,whichonlyexcises
˜

850minor/U12introns,distributedin750(U12) genes in the human genome. Mutations in the small nuclear RNA U4atac, an essential com- ponent of the minor spliceosome encoded by the RNU4ATAC gene, lead to rare neurodevelopmental syndromes mainly characterised by microcephaly, osteodysplasia and growth retardation.

To gain insights into the poorly-known physiopathological mechanisms of the RNU4ATAC- opathies, we generated morpholino (MO)-mediated u4atac zebrafish models and expressed human wild-type or mutated U4atac. Loss of function (LOF) of u4atac (MO or mutation) leads to microcephaly and cilium-related developmental defects, and to identify the U12 genes contributing the most to these phenotypes, we performed a bulk short-read RNA se- quencing on zebrafish heads of 2 day-old embryos. Principal component analysis based on intron retention (IR) showed three clusters: controls (control MO and WT hU4atac rescue), u4atac MO and mutated hU4atac rescue. 60 to 96% of U12 introns of expressed genes were significantly retained in u4atac-deficient conditions, with more than half of them having strong degree of IR (ΔIRratio > 10%). This result underlines the massive impact of u4atac LOF on U12 intron splicing. Nevertheless, only 11% of the U12 expressed genes exhibited differential expression (fold change > 2) compare to controls, indicating that the transcripts with U12 IR are barely subjected to RNA decay.

Surprisingly, beyond the impact on U12 intron splicing, mutated hU4atac expression leads to splicing alteration of 1% of U2 expressed introns: these introns appear better spliced in mutated conditions than in controls, suggesting that minor spliceosome also regulates alter- native splicing of major introns.

Finally, by crossing this dataset to those on human cells, we found 90 common genes with U12 IR, and chose to focus on two cilium-related genes, RFX7 and TMEM107, for further investigation. We confirmed U12 IR in both human and zebrafish models by RT-qPCR, and performed LOF and epistasis experiments in zebrafish, which validated the involvement of these genes, together with u4atac, in microcephaly

Structural basis of the U12-dependent intron recognition

Zhao Jiangfeng [1], Peter Daniel [1], Liu Xiangyang [1], Brandina Irina [1], ***Galej Wojtek [1]***

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The minor spliceosome catalyzes the excision of U12-dependent introns from pre-mRNAs. These introns are rare, but their removal is critical for cell viability. While the structure of the major spliceosome has been studied extensively, the architecture of the minor spliceosome remains largely unknown. Here, we report two cryoEM reconstructions of the apo- and substrate-bound U11 snRNP complexes, providing new insights into U12-type 5'SS recognition. Our structures reveal how the U11 snRNA binds five minor spliceosome-specific factors. U11 snRNP appears strikingly different from the equivalent major spliceosomal U1 snRNP. Unique interactions between U11 snRNA stem loops provide a structural basis for its specific recognition by the SNRNP25 and SNRNP35. Our substrate-bound structures reveal a unique bipartite 5'SS recognition mechanism by the U11 snRNP and the role of protein factors in stabilizing this interaction.

Taken together, our results provide new mechanistic insights into U12-dependent intron recognition and the evolution of the splicing machinery.

Transcription and mRNA processing recovery after global Pol II promoter proximal blocking

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We have recently developed technology to isolate authentic intact nascent transcripts associated with transcribing RNA polymerase II (Pol II) complexes, called POINT technology (Sousa-LuÃ_s at al., 2021). This has allowed us to isolate and study pre-mRNA undergoing mRNA processing, without the use of potentially interfering modified nucleotides. We have now combined our POINT technology with the Padgett technique that exploits the reversibility of the CDK9 inhibitor DRB (see also poster #XX). Upon DRB treatment, Pol II is blocked (synchronised) at proximal promoter pause sites. DRB washout then allows transcription to restart from the same sites in all cells, so that pre-mRNA begins to be produced. A time course of Pol II release from proximal promoter pausing (by DRB washout) affords the study of both co-transcriptional splicing and 3'end processing. We observe that whilst transcription recovers quickly after DRB washout, splicing and 3'end cleavage need more time to restart. This also causes a substantial transcriptional readthrough effect. We further demonstrate that DRB does not directly interfere with the splicing reaction. Instead, long DRB-treatment affects Pol II CTD phosphorylation patterns, which in turn prevent spliceosome recruitment to elongating Pol II complexes. Notably, we identify an increase in CTD Y1P after DRB washout, concomitant with splicing deficiency. Subsequent Mass Spec analysis shows that spliceosome components do not interact with Y1P Pol II. This suggests a possible role for Y1P in defining spliceable regions in transcription units. To conclude, our work shows that long DRB treatment, followed by a withdrawal of the drug uncouples transcription and mRNA processing.

Repertoire of mRNAs with trimethylated caps in mammals and study of their translation

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RNA modifications play important roles in the regulation of gene expression. Canonical cap-dependent translation relies on the presence of a 7-methylguanosine (m7G cap) at the 5' end of mRNAs to recruit the initiation factor eIF4E. The strict dependence on the eIF4E/m7G interaction is called into question by multiple transcriptomic analyses, challenging the classical cap-dependent translation model. We reported the first example of epitranscriptomic modification of the cap of certain mRNAs in mammals and showed that several stress-related selenoprotein mRNAs have a hypermodified m32,2,7G cap (or TMG cap)1,2. This modification is achieved by the trimethylguanosine synthase 1 (Tgs1). These TMG-capped mRNAs are not recognized by eIF4E but are nevertheless translated1. In contrast, the initiation factor eIF3 preferentially interacts with TMG-capped selenoprotein mRNAs rather than m7G-capped mRNAs3. Here, we identified the entire repertoire of TMG-capped mRNAs at the transcriptome level in HEK293FT cells. TMG immunoprecipitation coupled to RNA-seq (TMG RIP-seq) allowed us to identify more than 300 new TMG-capped mRNAs primarily involved in translation, stress response, antioxidant protection, and cancer prevention. Among them, mRNAs encoding ribosomal proteins, translation initiation factors, mitochondrial respiratory chain proteins, RAS chaperones, modulators of ER-stress, and regulators of mTOR signaling that contribute to cancer progression. Knock-down of Tgs1 by RNAi in HEK293FT cells resulted in reduced TMG-IP efficiency for these mRNAs, demonstrating that Tgs1 was responsible for the hypermethylation of their caps. A selection of synthetic TMG-capped or m7G-capped mRNA transcripts were used to decipher their translation mechanisms in vitro using HEK293FT or RRL cell-free translation assays. Results show that TMG-capped mRNAs can be translated in vitro, although with lower efficiency than their m7G-capped counterpart, and that their 5'UTR regions contain translation regulation elements. Our studies have extended the repertoire of TMG-capped mRNAs and aim to determine whether epitranscriptomic modifications of the cap can direct mRNAs towards alternative translation mechanisms. a 1. Wurth L. et al. (2014) Nucleic Acids Research, 42, 8663a 867. 2. Gribling-Burrer A.S. et al. (2017) Nucleic Acids Res, 45, 5399a 5413. 3. Hayek H. et al. (2022) Biomolecules, 9, 1268

Two microbiome metabolites competing for tRNA modification impact mammalian cell proliferation and translation quality control

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Microbiome interacts with the eukaryotic host through many metabolites to affect cell physiology. A direct molecular pathway of microbial-host interaction is the incorporation of the microbial metabolite queuine at the wobble anticodon nucleotide of host tRNAs (queuosine) by a host enzyme and regulating the host cell translation. Microbes also produce the intermediary metabolite pre-queuosine1 (preQ1) in the queuosine biosynthesis pathway, but how preQ1 can affect the host cell biology has not been explored. Here we show that preQ1 strongly represses human and mouse cell proliferation, however, this effect is suppressed or reversible with queuine and depends on the same host enzyme that installs queuosine tRNA modification. PreQ1, like queuine, is present in the plasma and tissues of mice and is incorporated into tRNAs in both in vitro mammalian cells and in mice. However, unlike queuine, preQ1 treatment suppresses tumor growth in a mouse cancer xenograft model. Mechanistically, preQ1 reduces cognate tRNA levels specifically and translation of house-keeping genes in a highly codon dependent manner. We identify the inositol-requiring enzyme 1 (IRE1) ribonuclease as the enzyme responsible for the selective degradation of preQ1-modified tRNAs on the translating ribosomes. Our results show an inter-dependent relationship of two microbial metabolites that regulate host cell proliferation through their tRNA modifications.

Exploring a Novel Class of Flavoenzymes: Identification and Biochemical Characterization of Ribosomal RNA Dihydrouridine Synthase.

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Dihydrouridine (D) is a prevalent and evolutionarily conserved modification found mainly in tRNAs and, to a lesser extent, in mRNAs. In E. coli, it extends to position 2449 of the 23S rRNA, strategically located in the ribosome's peptidyl transferase center. Despite the existence of known dihydrouridine synthases (DUS), which utilize NADPH and FMN, the enzyme responsible for biosynthesizing D2449 has remained elusive. The presented study introduces a rapid method for detecting D in rRNA, involving reverse transcriptase blockage at the rhodamine-labeled D2449 site followed by PCR amplification (RhoRT-PCR). Through analysis of rRNA from diverse E. coli strains, including those with chromosomal deletions and point mutations, the yhiN gene was pinpointed as the ribosomal dihydrouridine synthase, now designated as RdsA. Biochemical characterizations revealed RdsA as a novel class of flavoenzymes dependent on FAD and NADH, exhibiting a complex structural topology. In vitro assays demonstrated that RdsA dihydrouridylates an RNA transcript, mimicking a segment of the peptidyl transferase center, suggesting an early introduction of this modification before ribosome assembly. Phylogenetic studies unveiled the widespread distribution of the RdsA gene in the bacterial kingdom, emphasizing the conservation of rRNA dihydrouridylation. These findings underscore nature's preference for utilizing reduced flavin in the reduction of uridines and their derivatives, highlighting the importance of this modification in RNA biology and bacterial physiology, offering new paths for exploring the biological significance of PTC dihydrouridylation in ribosomal function.

The Dihydrouridine synthase DusB : a role in bacterial adaptation to oxidative stress

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tRNA modifying-enzymes regulate cellular processes, influencing factors such as tRNA folding, stability and decoding efficiency. Recent research additionally unveiled a moonlighting function as tRNA chaperone for 2 bacterial tRNA modifying-enzymes, namely TruB and TrmA. The dihydrouridine synthase B (DusB) catalyzes uridine (U) reduction to dihydrouridine (D) in the D-loop of tRNAs. This study investigates the role of V. cholerae DusB in bacterial adaptation to oxidative stress. We show that dusB deletion significantly impairs V. cholerae survival to hydrogen peroxide (H2O2) treatment. We characterize VcDusB substrates and demonstrate that DusB globally influences codon decoding efficiency and translation, even at codons not decoded by DusB-substrate tRNAs. Proteomic analysis revealed a substantial decrease in protein abundance in aˆ†dusB under oxidative stress, confirming a global translational defect. Furthermore, the study of 6 DusB point mutants highlight that the role of DusB in H2O2 survival is independent of its ability to catalyze D17 modification but instead relies on its tRNA binding capacity and NADPH oxidase activity. These findings underscore the critical role of DusB in in V. cholerae adaptation to oxidative stress, suggest new function(s) of DusB, and provide new insights into the mechanisms of bacterial adaptation under stressful conditions.

m6a methylation orchestrates IMP1 regulation of microtubules during human neuronal differentiation

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Neuronal differentiation requires building a complex intracellular architecture, and therefore the coordinated regulation of defined sets of genes. RNA-binding proteins (RBPs) play a key role in this regulation. However, while their action on individual mRNAs has been explored in depth, the mechanisms used to coordinate gene expression programs shaping neuronal morphology are poorly understood. To address this, we studied how the paradigmatic RBP IMP1 (IGF2BP1), an essential developmental factor, selects and regulates its RNA targets during the human neuronal differentiation. We perform a combination of system-wide and molecular analyses, revealing that IMP1 developmentally transitions to and directly regulates the expression of mRNAs encoding essential regulators of the microtubule network, a key component of neuronal morphology. Furthermore, we show that m6A methylation drives the selection of specific IMP1 mRNA targets and their protein expression during the developmental transition from neural precursors to neurons, providing a molecular principle for the onset of target selectivity.

The impact of RNA modifications on the translation fidelity

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Like DNA and proteins, RNA is subject to chemical modifications that can alter RNA metabolism like folding, stability, splicing, nuclear export, location and translation. Over a hundred chemical modifications have been identified in RNA, mostly in tRNA and rRNA but also in mRNA. However, the role of these modifications on translation is still unclear. In particular, the impact of mRNA and tRNA modifications on recoding events such as readthrough, during which tRNAs are incorporated at the stop codon, allowing translation to continue until the next stop codon, has not been investigated yet. To test the impact of mRNA modifications on translation accuracy, we are synthesizing mRNAs based on Glutathione S-Transferase (GST) sequence, carrying a stop codon in a readthrough context at the beginning of the coding sequence. In this way, GST is only produced after a readthrough event. These mRNAs are constructed in several parts. The region including the modified base is chemically synthesized; the rest of the sequence is in vitro transcribed. The two fragments are then ligated together to form the complete GST mRNA sequence. The readthrough efficiency is estimated with the quantity of the GST proteins produced in presence or absence of modification. The GST proteins are then analyzed by mass spectrometry to identify any change in amino acids due to the modified nucleotide. tRNAs are the most highly modified RNAs in cells. However, it is difficult to assess the impact of modifications on the ability of tRNAs to decode the corresponding codon, because in most cases, cognate tRNAs are efficiently incorporated regardless of their modification. To circumvent this problem, we chose to study the incorporation of tRNAs at a stop codon, during a readthrough event in human cells in culture. We could highlight the complex interrelationship between two modifications present in the anticodon loop of the human tyrosine tRNA. While Psi35 appears to be essential for the decoding of both tyrosine codons and UAG/UAA stop codons by the tyrosine tRNA, we were able to show that the GalQ modification at position 34, which is specific to human cells, reduces the ability of tyrosine tRNA to be incorporated as a near-cognate. As the precursor of GalQ34 is mainly provided by the microbiota, this suggests an important role of the human microbiota in the decoding efficiency and translation fidelity.

Circular RNAs: new players in the dialogue between chemotherapy-resistant lung cancer cells and dendritic cells ?

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Circular RNAs, a new class of RNAs, have been shown to play a role in the initiation, progression and development of many cancers, including lung cancer. However, their role in therapy resistance remains to be investigated, and in particular their impact on the dialogue between cancer cells and immune cells in the tumor microenvironment, such as dendritic cells (DCs). A better understanding of the effects of circRNAs in this dialogue could lead to the identification of predictive biomarkers of response to chemo-immunotherapies widely used in the clinic. We have identified 3 circRNAs whose expression is significantly increased in lung cancer cells resistant to platinum salts, the reference chemotherapy, compared with sensitive cells. To investigate the role of these circular RNAs in the dialogue between cancer cells and DCs, co-cultures were developed and dendritic cell expression of activation markers and immune checkpoints was analyzed by flow cytometry. We show that circular RNAs expressed by resistant cancer cells influence the anti- or pro-tumor phenotype of dendritic cells, as well as the secretion of soluble factors by DCs and cancer cells. Our results also show that the expression of these circular RNAs in cancer cells impacts the NFkB pathway, an important modulator of the tumor microenvironment. Furthermore, these circular RNAs are detected in liquid biopsies from lung cancer patients, and their quantity is impacted by the chemotherapy received by the patients.

Aptamer binding footprints (AptaFOOT-Seq) to improve the diagnosis of synucleinopathies.

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Synucleinopathies, such as dementia with Lewy bodies (DLB), Parkinson’s disease (PD), and multiple system atrophy (MSA), are characterized by the accumulation of α-synuclein (α-syn) aggregates in the central nervous system. Emerging evidence suggests that the clinical and pathological variability of these disorders may be due, in part, to the presence of distinct α-syn fibrillar polymorphs, each exhibiting unique structural features. One promising approach to study and detect these polymorphs is the use of conformation-specific ligands that can selectively bind to different α-syn fibril structures.

In this study, we identify nuclease-resistant 2′fluoro-pyrimidine RNA aptamers capable of differentially binding to various α-syn fibrillar polymorphs. We also introduce a novel method, AptaFOOT-Seq, which utilizes next-generation sequencing to rapidly assess the binding affinities of aptamer mixtures for different α-syn polymorphs. Our findings demonstrate that the behavior of aptamers varies significantly when tested individually versus in a mixture, where interactions such as competition and cooperation occur. These interactions generate unique binding "footprints" that can differentiate between α-syn fibrillar polymorphs derived from patients with PD, DLB, or MSA. This suggests that AptaFOOT-Seq has the potential to detect misfolded protein conformations, offering a novel diagnostic tool for synucleinopathies.

Rational Design of RNA-Based Therapeutics for Cancer Treatment

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Cell carcinogenesis is a complex process that triggers significant changes in gene expression, leading to uncontrolled cell proliferation. The RNA-binding protein RBM39 (RNA Binding Motif 39) has been identified as essential for the survival of various cancer cells. Depleting RBM39 through genomic methods (CRISPR/Cas) or pharmacological means (aryl sulfonamides) results in the death of these cancer cells, establishing RBM39 as a validated anti-cancer target. Aryl sulfonamides act as molecular glues, facilitating the association of RBM39 with the protein DCAF15, which is linked to ubiquitin ligase activity, leading to the targeted degradation of RBM39. However, clinical trials indicate that aryl sulfonamides are effective in only 16-30% of patients, with this response rate positively correlating with DCAF15 abundance. Therefore, new approaches are needed to eliminate RBM39 from cancer cells independently of DCAF15. In this context, we have recently elucidated the molecular basis of RBM39's specific RNA recognition and a negative feedback mechanism enabling RBM39 autoregulation at the RNA splicing level. Our project aims to leverage this knowledge to develop RNA therapeutics that can reduce RBM39 amount within cancer cells and induce their death. We are employing three strategies: (i) a siRNA based strategy; (ii) an antisense RNA approach to manipulate the autoregulation mechanism of RBM39, and (ii) an RNA bait approach to inhibit RBM39 activity. During this talk, I will present the results from the three approaches. The RNA therapeutics were developed in model cell lines to observe their effects on RBM39-dependent RNA splicing events and their anti-cancer activities was measured in colorectal cancer cell lines. The results are promising, as the RNA therapeutics effectively kill colorectal cancer cells at very low doses and in a dose-dependent manner. We are now advancing toward the in vivo evaluation of these RNA therapeutics

Unlocking the Future of RNA based vaccines: Tebubio's production solutions

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Since the Covid-19 pandemic, the application of RNA vaccines for cancer treatment has represented significant hope, although clinical effectiveness remains to be demonstrated. To achieve this, researchers require high-quality production of vaccine mRNA and accurate, cost-effective testing tools tailored to oncology research. At Tebubio, we have developed a complete pipeline to meet researchers needs. Our Contract Research Services offer customizable RNA production solutions, providing small quantities (from 100 µg to 2 mg) of Research-Grade mRNAs. Complemented by a formulation and RNA delivery platform using lipid nanoparticles (LNPs) and cellular tests with biomarker analysis. Through concrete examples, such as the analysis of the stability and expression of the tumor antigen p53 in antigen-presenting cells (APCs), or data on mRNA encapsulation within LNPs and expression analysis in different cellular models, find out how Tebubio can help you speed up your research projects.

Overview of Sanofi's mRNA Center of Excellence (CoE) and example of characterization for new mRNA vaccines: RNA secondary structure prediction at different mRNA vaccine stages

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In 2021, Sanofi inaugurated a new center to work on mRNA vaccines. This center called mRNA Center of Excellence (mRNA CoE) is located on 2 countries France and the USA. The objective of this center is to accelerate mRNA technology for vaccines, therapeutics and innovation. For that more than 600 employes are working in an end-to-end platform comprising R&D unit to manufacturing. One of the innovation and R&D work is on the determination of RNA secondary structure at different mRNA vaccines stages.

Messenger RNA (mRNA) vaccines contain synthetic mRNAs that code for target antigens to induce an immune response. To be effective, mRNAs must reach target tissues and be internalized by cells to be translated into proteins by cell machinery in the cytoplasm. To protect nucleic acid from potential degradation, a stable delivery system is required, such as lipid nanoparticles (LNPs), which have been widely used for the COVID-19 vaccine. LNPs allow mRNA to be encapsulated and protected, thanks to several types of lipids that assemble spontaneously1. During the formulation process, mRNA is in solution in different buffers whose composition varies. These conditions can influence the mRNA structure, which is a very flexible and dynamic molecule that can adopt various structures (secondary and even tertiary).

This secondary structure consists in the combination of different patterns such as helix, loops that are interconnected by junctions. The characterization of this structure can be carried out using biophysical techniques such as Cryo-EM but also by biochemical techniques called "RNA structure probing". Biological macromolecules (RNAses) or small chemical molecules can probe the structure of RNA by identifying nucleotides in single strand conformation2. We decide to use the SHAPE (Selective 2'-OH acylation analyzed by primer extension)3 method to understand and characterize structural variations of a mRNA that can be induced during the process of formulation of mRNA into LNP.

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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 [1]. ADAR1 has been proposed to form a stable homodimer and dimerization is suggested to be important for editing activity [2]. 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 [3]. 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 [4,5]. [1] Walkley CR et al. (2017) Rewriting the transcriptome: adenosine-to-inosine RNA editing by ADARs. Genome Biol 18:205.
[2] Cho DC et al. (2003) Requirement of dimerization for RNA editing activity of adenosine deaminases acting on RNA. J Biol Chem 278:17093-102.
[3] Mboukou A et al. (2023) Dimerization of ADAR1 modulates site-specificity of RNA editing. bioRxiv 570066.
[4] Ishizuka JJ et al. (2019) Loss of ADAR1 in tumours overcomes resistance to immune checkpoint blockade. Nature 565:43-48.
[5] Datta R et al. (2024) A-to-I RNA editing by ADAR and its therapeutic applications: From viral infections to cancer immunotherapy. Wiley Interdiscip Rev RNA 15:e1817.

Microprotein-coding intronic polyadenylation isoforms: A new genetic paradigm

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On the one hand, many human genes generate intronic polyadenylation (IPA) isoforms, that terminate in an alternative last exon and often encode isoforms of canonical proteins. On the other hand, microproteins are an emerging class of small proteins translated from small open reading frames (sORFs) located in annotated noncoding RNAs and canonical mRNAs. However, microprotein production by IPA transcripts has not been studied. In this study, based on multiple omics analyses (3'-seq on total RNA and polysome fractions, short- and long-read RNA-seq, Ribo-seq and mass spectrometry), we reveal that IPA isoforms are a novel source of microproteins. Indeed, we show that many human protein-coding genes generate a microprotein-coding IPA transcript (coined miP-5'UTR-IPA isoform), from the same promoter as the canonical mRNA but using a polyadenylation site located in an intron within the annotated 5'-untranslated region of the gene. By 3'-seq on lung cancer cells, we show that cisplatin (a genotoxic agent commonly used to treat this cancer) favors the expression of many miP-5'UTR-IPA isoforms relative to matched canonical mRNAs, through an inhibition of transcription processivity in a FANCD2 and senataxin-dependent manner. We further characterized the miP-5'UTR-IPA isoform of one of these genes, PRKAR1B: we detected the encoded microprotein; CRISPR editing of either the IPA site or the sORF initiation codon in A549 cells led to a decrease of cell growth inhibition by cisplatin; motif analysis in the microprotein predicted its interaction with two p53-regulatory proteins; and accordingly, CRISPR clones showed decreased p53 protein induction by cisplatin. Finally, we are developing a database of human miP-5'UTR-IPA isoforms and show their expression in normal tissues and cell types. Altogether, these findings reveal the novel paradigm of miP-5'UTR-IPA genes, provide the proof of principle of their functionality and suggest their role in cancer cell response to a genotoxic agent.

Tau mutations and short 5'UTR tau mRNAs boost tau translation

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Tau protein plays a central role in Alzheimer's disease (AD) and other neurodegenerative diseases termed Tauopathies. Tau protein is known to aggregate, and its expression is important for disease development and progression. The generation of truncated Tau species is also involved in the pathological process. Interestingly, several 5'end truncated Tau mRNAs were found overexpressed in brains with tauopathies. In addition, N-terminal mutations like R5H, R5L and R5C were associated with AD, progressive supranuclear palsy (PSP) and Parkinson's disease (PD), respectively. However, the mechanisms that lead to truncated Tau species and the relevance of N-terminal mutations for the diseases remain largely unclear. We aimed to investigate how the 5'end truncated Tau mRNAs and N-terminal mutations impact on the production of different Tau species. To achieve this, we synthesized tau mRNAs with different 5'UTR lengths and/or containing disease-causing mutations. These transcripts were used to monitor Tau translation in cell-free translation extracts from rabbit reticulocyte lysates (RRL), HEK293T cells and SH-SY5Y cells. Also, we assessed the RNA structure by chemical probing with DMS/CMCT. Our work shows that the length of the 5'UTR of Tau mRNAs and the disease-associated N-terminal mutations largely impact on its translation. Also, the same mRNA can generate truncated Tau species by an alternative translation initiation event. Additionally, these mutations induce changes in the secondary structure of Tau mRNA. Altogether, our work shows that Tau mRNA identity around the 5' end is critical to regulate its translation initiation. This underestimated source of Tau protein species might contribute to the development of Tauopathies.

ZZEF1 drives a metastasis-suppressing tRNA expression program in breast cancer

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Human genome contains hundreds of transfer RNA (tRNA) genes whose expression is cell type- and context-specific. How this specificity is attained remains largely unknown as no tRNA gene-specific transcription factors have been identified so far. Here, by applying computational and experimental tools we show that ZZEF1 is necessary for a sustained tRNA-LysUUU expression, and subsequently for the translation of the high lysine content proteins. ZZEF1 acts with CHD6 to enhance chromatin accessibility at the target tRNA loci. In breast cancer, lower ZZEF1 levels lead to increased metastatic dissemination in xenograft mouse models, and is associated with poor patient survival in clinical datasets. We identify STK3, a lysine-rich protein kinase, acting downstream of the ZZEF1-tRNA-LysUUU axis to promote metastasis.

From the last intron to the 3'UTR: a high number of variants in/near MSH2 terminal exon alter RNA splicing of this tumor suppressor gene

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The interpretation of genetic variants located at terminal exons or their adjacent intronic regions remains a major challenge in medical genetics, as their effects on RNA and/or protein are often difficult to predict. This is notably the case for the last exon (ex16) of MSH2, a tumor suppressor gene implicated in Lynch Syndrome (LS). Identification of the causal variant is critical for LS diagnosis and optimal medical care. Yet, numerous MSH2 ex16 variants remain classified as of uncertain significance (VUS) and cannot be used to inform clinical decisions. Here, we report results from functional analyses on variants mapping to MSH2 ex16 or to its adjacent intronic region (in15). Our methods included analysis of patients' RNA by RT-qPCR, cell-based minigene splicing assays and assessment of MSH2 function by performing a methylation tolerance (MT) assay on MSH2-/- cells transiently expressing MSH2 cDNA. RT-qPCR analysis of patients' RNA revealed that 5 out of the 6 variants evaluated in this context (4 intronic and 2 exonic variants) were associated with a decrease in canonical ex15-ex16 splicing and an increase in alternative ex15-ex17 junction. These results suggest that ex17 (a poorly expressed MSH2 alternative terminal exon) can be used as a reporter in patient RNA analyses to reveal the presence of spliceogenic variants within in15/ex16. Importantly, our minigene splicing assays confirmed that the above mentioned spliceogenic variants do have a negative impact on ex16 splicing, validating our minigene construct to study additional variants especially when patient's RNA is unavailable. In total, we have now analyzed 62 in15/ex16 variants in the minigene assay (10 intronic and 52 exonic), out of which 43 showed a negative impact on splicing. Overall, our findings reveal an unexpected high fraction of spliceogenic variants in/near MSH2 ex16, including not only intronic variants within/near the 3'ss, but also missense, synonymous, truncating and 3'UTR variants. In addition, we performed a MT assay to better characterize, from the protein point of view, the most terminal nonsense variants identified in ex16 currently classified as VUS. These experiments (i) revealed which of these variants alter MSH2 DNA damage response activity and (ii) brought clues as to the minimal C-terminal sequence required for protein's function. We are currently collecting clinical, tumor and family data associated to the variants of interest, in order to aggregate arguments for classifying them clinically according to ACMG/NGS-Diag recommendations. This study underlines the importance of using complementary experimental approaches, at both RNA and protein levels, to better interpret variants mapping to a terminal exon.

Structural studies of nuclear cap-binding complexes shed light on fate determination mechanism of RNA Pol II transcripts.

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Eukaryotic gene expression requires precise co-transcriptional sorting of nascent RNA toward maturation, transport or degradation. The nuclear cap-binding complex (CBC), with its partner ARS2, forms mutually exclusive complexes with diverse RNA effectors, promoting either of these productive or destructive outcomes for Pol II transcripts. To better understand the molecular details underlying this RNA fate determination mechanism, we structurally and biochemically characterized how several effectors interact with the CBC-ARS2 complex. We show that the snRNA export factor PHAX, the mRNA export factor NCBP3, and the RNA-targeting NEXT complex subunit ZC3H18 compete for CBC binding via a conserved tryptophan-containing helix, and for ARS2 binding via a conserved ARM motif. For ZC3H18, we demonstrated in vivo that both ARS2 and CBC interaction are required for nuclear RNA degradation. In addition, our biochemical data showed that ARS2 is responsible for the initial effector recruitment but inhibits their direct binding to CBC until irreversible fate determination. Finally, our recent structural and biochemical work reveals how PHAX governs the formation of the snRNA export complex composed of CBC-PHAX-CRM1-RanGTP, leading to the competitive displacement of ARS2 and other RNA effectors from CBC, thus promoting a productive RNA fate.

Multi-omics and genetics analyses of pat1-deficient yeast cells uncover a transcriptome-wide impact on poly(A)-tail length suppressed by a ribosomal protein mutation

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Eukaryotic mRNAs are key actors of gene expression; thus, their levels need to be finely tuned. Post-transcriptionally, levels of mRNAs are governed by their turnover in the cytoplasm. Critical steps of mRNA decay encompass deadenylation, decapping, and exonucleolytic mRNA body digestion, that constitutes the 5' -> 3' pathway. The conserved decapping regulator Pat1 appears to impinge on all those processes. Despite evidence that Pat1 is a conserved and central actor of the 5' -> 3' mRNA decay pathway, some studies have reported that only a fraction of the transcriptome is impacted by its deletion. Moreover, Pat1 was described both as a translational repressor of oligoadenylated mRNAs and as a global translation initiation enhancer. Altogether, published data fail to provide definitive clues about the role of Pat1 in cellular mRNA control. To elucidate Pat1's function, we performed multi-omic and genetic analyses of a Δpat1 mutant in the yeast Saccharomyces cerevisiae. Transcriptome and proteome analyses revealed that the expression of only a subset of genes is affected by Pat1's inactivation. In contrast, a FLEP-seq analysis, allowing the sizing of the poly(A)-tail of each transcript in the cells by Nanopore sequencing, revealed that the absence of Pat1 impacts the length of the poly(A)-tail at the transcriptome level. Unexpectedly, we have observed that the growth defects caused by Pat1's inactivation are partially suppressed by a mutation in a ribosomal protein. This suppressor mutation also partly rescued the abnormal poly(A)-tail length of Δpat1 cells. Moreover, we observed that polysome distributions are altered in the absence of Pat1 and further modulated by the suppressor. Altogether, our analyses are consistent with the model where the activation of decapping, that becomes rate-limiting, is the primary defect in the absence of Pat1. Our study supports the conclusion that Pat1 is a general mRNA decay factor even if its absence results in transcript-specific changes in mRNA levels. Altered mRNA decay in Pat1 deficient cells, and consequent translational impairments, can both be partly rescued by altered ribosomes.

An unexpected role of the NMD machinery in targeting oligoadenylated mRNAs for degradation

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The nonsense-mediated mRNA decay (NMD) is a conserved eukaryotic cytoplasmic surveillance pathway that degrades aberrant mRNAs carrying premature termination codons (PTC). In yeast, the long distance between the poly(A) tail and the stop codon dictates mRNA detection by the central NMD factor Upf1 and its partners Upf2 and Upf3. The Upf proteins trigger the decapping and the 5' to 3' degradation of the targeted mRNA, independent of prior deadenylation. Some evidence suggest that Upf1 recognizes mRNAs other than archetypical PTC-containing mRNAs but the whole spectrum of Upf1 targets and the associated molecular mechanisms remain unclear. By direct Nanopore mRNA sequencing, we investigated the nature of Upf1-bound mRNAs in depth. Interestingly, we observed that the expected NMD substrates harbored long poly(A) tails, validating the deadenylation-independent degradation model of NMD. Strikingly, the largest fraction specifically associated to Upf1 corresponded to oligoadenylated mRNAs, a subfraction of many stable mRNAs likely at a particular stage of their life. Despite the absence of a PTC, the stability of these oligoadenylated mRNAs depends on the three Upf NMD factors. These results unveil a hitherto unknown major function of Upf1 and NMD cofactors in oligoadenylated mRNA decay.

Cell cycle-dependent mRNA localization in P-bodies

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Understanding the dynamics of RNA targeting to membraneless organelles is essential to disentangle their functions. Notably, whether p-bodies (PBs) participate in regulation of gene expression remains unclear. To tackle this question, we investigate how PBs evolve during cell cycle progression. PB purification across the cell cycle uncovers widespread changes in their RNA content, which are partly uncoupled from cell cycle-dependent changes in RNA expression. Single molecule FISH shows various mRNA localization patterns in PBs peaking in G1, S, or G2, with examples illustrating the timely capture of mRNAs in PBs when their encoded protein becomes dispensable. However, rather than directly reflecting absence of translation, cyclic mRNA localization in PBs is controlled by extrinsic (RNA binding proteins) and intrinsic (RNA features) factors. Indeed, while PB mRNAs are AU-rich at all cell cycle phases, they are specifically longer in G1, suggesting their involvement in post-mitotic PB reassembly. Altogether, our study supports a model where PBs are more than a default location for excess untranslated mRNAs.

Control of small RNA dynamics during programmed DNA elimination in Paramecium

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Ciliates undergo developmentally programmed DNA elimination, in which small RNAs direct the removal of transposable elements during the development of the somatic nucleus. 25-nt scnRNAs are produced from the entire germline genome and transported to the maternal somatic nucleus, where selection of scnRNAs corresponding to germline-specific sequences is thought to take place. Selected scnRNAs then guide the elimination of transposable elements in the developing somatic nucleus. How germline-specific scnRNAs are selected remains to be determined. Here, we sequenced small RNAs during the entire sexual cycle with unprecedented precision. Our data confirmed that scnRNAs are produced from the entire germline genome, from germline-specific sequences and somatic-destined sequences, during meiosis. We provided important mechanistic insight into the scnRNA selection pathway by identifying a set of proteins as essential for the selective degradation of scnRNAs corresponding to somatic-destined sequences. By immunoprecipitation coupled with quantitative label-free mass spectrometry, we found that all the identified proteins associate with the scnRNA binding Ptiwi09 protein. Functional characterization of these factors is consistent with the idea that selective degradation of scnRNAs occurs in the maternal somatic nucleus and is critical for DNA elimination. Altogether, our data suggest that the pairing between scnRNAs and somatic non-coding RNA leads to the coordinated degradation of the Ptiwi09 protein and its bound scnRNAs, similarly to the proposed mechanism for microRNA target-directed degradation.

Embedding a ribonuclease in the spore crust couples gene expression to spore development in Bacillus subtilis

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Some firmicutes can adapt a highly resistant and dormant bacterial state called an endospore, that allows them to survive extreme environmental conditions for millions of years (1-4). The agents of anthrax, botulism or tetanus have integrated spore formation into their pathogenic cycle, making this a critical cell development process for medical research. Bacterial RNA degradation is both important for the recycling ribonucleotides and controlling gene expression through a variety of mechanisms (5,6). The activity of some ribonucleases and the accessibility of their RNA substrates are regulated under specific stress conditions, such as nutrient starvation or amino-acid depletion, to allow a tight control of RNA decay and maturation (5,7). Yet, the role of ribonucleases in the sporulation processes has not been studied in much detail. Although some RNases are required for sporulation to occur, their mechanisms and function during sporulation are poorly understood (8-10). Here we present KapD, a ribonuclease exhibiting a 3'-exonuclease activity and specifically expressed during sporulation of B. subtilis. KapD is regulated by the sporulation specific sigma factors E and K, allowing expression within the mother cell at the early and late stages of sporulation (2). Deletion of the kapD gene or inactivation of its catalytic site strongly decreases the adhesiveness of the outer coat and crust layers of the endospore, suggesting that its ribonuclease activity is essential for the correct formation of the spore morphological structure. Using dual-color fluorescent microscopy, we show a dynamic assembly of the protein into the spore crust layer in a similar way to outer spore coat proteins. Through yeast two-hybrid assay and in vitro validation, we showed that KapD interacts with the well-known crust protein CotY, accounting for its localization in the outermost spore layer. This interaction inhibits the catalytic activity of KapD. Coupling proteomic analysis with Northern blotting, we identified a key developmental transcriptional regulator sigK mRNA as the main substrate of KapD. In fine, we provide evidence that KapD controls the timing of expression and activation of SigK prior to its sequestration within the spore crust layer, to couple spore morphology to sigK gene expression and add an extra layer level of regulation to the the control of the expression of this key sigma factor. (1) Riley, E P ; et al. (2020) Microb Cell. 8(1) :1-16 (2) Pereira, F C ; et al. (2013) PLoS Genet. 9(10) : e1003782 (3) Vreeland, R H; et al. (2000) Nature. 407(6806) :897-900 (4) Cano, R J & Borucki, M K. (1995) Science. 268(5213):1060-4 (5) Condon, C. (2007) Curr Opin Microbiol. 10(3):271-8 (6) Condon, C; et al. (2021) C R Biol. 344(4) :357-371. (7) Cook, G ; et al. (2013) Biochim Biophys Acta. 1829(6-7) :523-31 (8) Sello J.K ; et al. (2008) J. Bacteriol. 190(11) :4079-83 (9) Figaro, S. et al (2013) J. Bacteriol. 195(10) :2340-8 (10) Myers T M ; et al. (2023) Nucl. Ac. Res. 51(18) : 9804-9820

The CCR4-NOT complex and codon optimality promote co-translational mRNA targeting to the ER

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In eukaryotes, up to a third of the proteome is translocated to the ER and this is critical for proper protein localization, folding and post-translational processing of most membrane, secreted, ER and Golgi proteins. Most of these proteins are co-translationally translocated into the ER. The canonical model for co-translational ER targeting involves the recognition by the SRP of a hydrophobic peptide emerging from the ribosome exit tunnel. SRP-bound ribosomes are then addressed to the ER, where the nascent chain is elongated concomitantly with its translocation into the ER. However, ER-specific ribosome profiling experiments in yeast showed that some mRNAs can be co-translationally targeted before the hydrophobic signal exits the ribosome, in an SRP-independent manner. This observation suggests that mRNA sequences carry information for ER-targeting. We previously showed that CNOT1, the scaffold subunit of the CCR4-NOT complex, is necessary for the translation and localization of ER-targeted mRNAs. CCR4-NOT can be recruited to specific mRNAs through miRNAs and RBPs but also by directly sensing codon optimality via its CNOT3 subunit. By combining subcellular fractionation and RNA sequencing, we showed that non-optimal codons are more frequent in membrane-bound transcripts in human cells. Transcripts with lowest codon optimality dissociated from the ER when silencing CNOT1 or CNOT3. Furthermore, we observed that leucine CUG codons are enriched in ER-targeted transcripts and more specifically in regions encoding hydrophobic signals. The frequency of this particular codon also correlated with ER dissociation upon CNOT1 or CNOT3 depletions. We showed that synonymous mutations of CUG codons in signal sequences of Gaussia Luciferase reporters disrupt their secretion without affecting their RNA levels. Finally, reanalysis of previously published datasets revealed that ER-targeting signals are also encoded by non-optimal codons in yeast, suggesting that this mechanism is conserved across species. Altogether, these results support a model where the CCR4-NOT complex, codon optimality and CUG codons promote co-translational ER targeting.

Quantitative mass spectrometry of affinity-purified ribosomes uncovers ribosome interactome specialization during viral infections

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Viruses depend on host ribosomes to translate their mRNAs and have evolved numerous mechanisms to hijack the cellular translational machinery. To gain a deeper understanding of how these intracellular parasites manipulate the translation apparatus, we developed an enhanced ribosome tandem affinity purification protocol (eRAP), coupled with formaldehyde cross-linking, to characterize the ribosome interactome through quantitative mass spectrometry. Using eRAP, we identified approximately 700 ribosome-associated proteins involved in diverse cellular functions, including mRNA metabolism, protein modification, the cell cycle, and metabolic processes. When applied to cells infected with Sindbis (a positive strand RNA virus from the Alphavirus genus), eRAP uncovered a profound perturbation of the ribosome interactome, including a strong decrease in factors associated with cap-dependent translation and ribosome biogenesis. In addition to disrupting the translation initiation machinery, Sindbis co-opts the host oligosaccharyltransferase complex to ribosomes for co-translational N-glycosylation of viral envelope proteins. Moreover, infection increases recruitment to polysomes of the dimer DENR-MCTS1 (a translation re-initiation factor) and ASC-1 (a complex involved in resolving ribosome collisions) and Sindbis relies on these complexes for efficient replication. Our findings suggest that DENR-MCTS1 may functionally replace eIF2 to drive translation of the viral subgenomic RNA upon PKR activation. Surprisingly, the role of ASC-1 in the context of Sindbis infection does not depend on canonical marks of collided ribosomes as previously described in the literature, but is rather linked to the regulation of co-translational protein modification events at the endoplasmic reticulum, which are required for the correct maturation of viral proteins. Finally, changes in ribosome-associated proteins upon infection are largely independent of alterations in RNA-binding proteins (as probed by RNA interactome capture), suggesting that these two regulatory pathways function separately. This study uncovers a new layer of gene expression regulation specific to ribosomes, and provides new insights in the complex relationship between viruses and their hosts.

Spatial organization of translation and translational repression in two phases of germ granules

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Biomolecular condensates are membrane-less compartments that allow the coordination and efficiency of biochemical reactions in the cell by concentrating substrates and enzymes in a confined space. They are formed by phase separation, a demixing process that results from multivalent interactions between proteins and nucleic acids. Among biomolecular condensates, RNA-Protein (RNP) condensates that are formed by RNAs and RNA Binding Proteins, are hubs for post-transcriptional regulation. Several types of RNP condensates are not homogeneous, but rather composed of several immiscible phases. However, how these different phases are linked to their biological functions remains a key question. Germ granules are RNP condensates essential for germ cell specification and differentiation, involved in the coordination of germ cell mRNA localization and translational regulation. They represent an outstanding model to study the relationships between the organization and functions of RNP condensates. We are using germ granules in the Drosophila embryo as a model. Using Stimulated-Emission-Depletion (STED) super-resolution microscopy, we showed that Drosophila germ granules have a biphasic organization comprising a shell and a core, with germ granule main protein components being enriched in the shell. We set up single-molecule imaging, including the Suntag approach to visualize translation taking place, and found that translation occurs in the shell and immediate periphery of the granule, but not in the core. Additionally, we followed the localization of sequentially translated mRNAs within germ granules and demonstrated a correlation between their translational status and their position. Translating mRNAs are enriched in the shell, whereas repressed mRNAs accumulate in the core. mRNA orientation and compaction within the granules also depend on their translation status: the 5'end of translated mRNAs are oriented towards the surface and these mRNAs adopt a less compacted conformation than repressed mRNAs. Finally, we found that altering germ granule structure severely affects mRNA translation levels. These findings reveal the importance of RNA granule architecture in organizing different functions, highlighting the functional compartmentalization of RNA granules.
A.R. and A.H. contributed equally to this work

Co-translational sorting enables a single mRNA to generate distinct polysomes with different localizations and protein fates

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β-catenin is a multi-functional protein playing essential roles in tissue homeostasis and cancer. It bridges E-cadherin to the cytoskeleton and also activates transcription in response to Wnt. Plasma membrane β-catenin is stable whereas without Wnt, cytoplasmic β-catenin is degraded by the destruction complex, composed of APC and Axin. Here, we show that APC and Axin associate with many mRNAs and that this occurs via the nascent protein chains. Notably, APC and Axin bind β-catenin mRNAs present as either single polysome or polysome condensates, and co-translational interactions constitute the major fraction of their binding to the β-catenin protein. Remarkably, E-cadherin also binds β-catenin co- translationally, and β-catenin mRNAs localize either with APC in the cytosol or E-cadherin at the plasma membrane. Thus, co-translational interactions sort β-catenin mRNAs into distinct polysome populations that spatially segregate in cells and synthesize proteins with different functions. Co-translational polysome sorting provides a mechanism to regulate the fate of multi-functional proteins.

30S-RiboSeq redefines the bacterial Ribosome Binding Site

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Translation initiation is a bottleneck of gene expression that determines translation efficiency. While this step is globally conserved, key aspects diverge between bacteria and eukaryotes. In the latter, the small subunit of the ribosome binds mRNA 5'-end caps and scans the mRNA to find the start codon, while in bacteria no scanning mechanism was described. Instead, the small subunit of the ribosome (30S) directly recognizes translation initiation regions, most often through the base-pairing of the 16S rRNA to the Shine Dalgarno (SD) region of mRNAs. However, in a few examples, loading events occur far within 5'UTRs, forming standby sites for ribosomes and showing that 30S loading does not occur exclusively at start regions. Together with the large number of degenerated SD sequences and of long mRNA 5'UTRs, this raises the possibility that 30S could bind away from the start codon much more commonly than currently thought. Hence, we sought to globally map for the first time the 30S-mRNA interactions in bacteria, here in Escherichia coli. For this, we developed the 30S-RiboSeq, based on the Translation Complex Profile sequencing (TCPseq) [1] used in Eukaryotes. A metagene analysis of our results recapitulates different 30S and 70S conformations during the translation initiation process. Furthermore, while 30S predominantly occupy start regions, our data remarkably show that an important population of 30S binds specific sites within 5'UTRs, sometimes over 100 nucleotides upstream of the SD region. We validated several of these newly identified binding sites by in vitro toeprinting assays, and further demonstrate their strong impact on gene expression. Hence, even in bacteria, ribosomes commonly bind mRNAs outside the start region to initiate translation, thereby challenging the classic ribosome binding site model. 1. Archer, S.K., Shirokikh, N.E., Beilharz, T.H., and Preiss, T. (2016). Dynamics of ribosome scanning and recycling revealed by translation complex profiling. Nature 535, 570–574. 10.1038/nature18647.

Glucocorticoid receptor activation improves dichotomy between translated and untranslated mRNA through active RNA storage in Processing-bodies

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Processing-bodies (P-bodies) are described as small cytoplasmic, membraneless organelles that play an important role in various cellular processes by controlling RNA translation. P-bodies are formed by the coalescence of untranslated mRNA and multiple RNA-binding proteins through liquid-liquid phase separation. Despite recent discoveries about their key components, the cellular pathways that control their formation are poorly understood. In this context, we performed a drug screen to identify targets that can promote P-body formation. We found that glucocorticoids (GC) can increase the number of P-bodies in epithelial cells within 48 hours of treatment. In addition, we showed, by genetic invalidation, that GR activation is required to increase the number of P-bodies upon GC treatment. Furthermore, we determined that only rescue of the GRa isoform was able to reproduce a change in P-body regulation in an inverted U-shape dose response, suggesting that both GC and GRa levels are important for triggering P-body reshaping. We evidenced that this phenotype is transient and reverts after GC removal. Finally, using transcriptomic and proteomic analyzes, we showed that, while some RNAs are less translated under GC, suggesting that there are stored in P-bodies, the correlation between translated mRNA and protein is enhanced. These results suggest that GR activation promotes a better dichotomy between RNA that “needs to be translated” and RNA that “needs to be silenced”. Overall, our results show that, in addition to its known transcriptional activity, GR is also able to influence global RNA storage and translation in different epithelial cell types.

The C19MC Gene Locus: Evolutionary Significance and Regulatory Functions in Placenta-Derived Cells

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The human C19MC gene locus consists of an approximately 100 kb-long non coding array of tandemly repeated, paternally expressed miRNA genes that are specific to primates. These miRNAs are only expressed in the placenta, where they make up 20-25% of the total miRNA population. Notably, around 15 of the 46 C19MC miRNAs share a conserved AAGUGC seed motif, which is also found in other miRNA families that emerged at various stages of vertebrate evolution. This includes the tetrapod-specific miR-302 cluster and the placental mammal-specific miR-290-295 cluster, both of which are crucial in early development, particularly in regulating pluripotency and the self-renewal of embryonic stem cells. This shared seed motif, also known as the "oncomiR," is also involved in silencing numerous tumor-suppressor genes. As a result, the C19MC locus holds significant evolutionary and functional importance, with potential roles in both developmental processes and tumorigenesis. However, its specific functions in placenta-derived cells remain relatively unexplored. To investigate the regulatory role of C19MC in placenta-derived cells, we used CRISPR-Cas9 to delete the active paternal allele in a choriocarcinoma (JAR) cell line. In these C19MC knockout (KO) cells, we observed altered epithelial integrity, including an increase in multinucleated cells. These morphological changes were accompanied by a reduced proliferation rate, an extended G1 phase and diminished migratory activity. Using next-generation sequencing techniques such as mRNA sequencing, polysome profiling and Ago2-crosslinking immunoprecipitation, we also identified several disrupted gene-regulatory pathways. This includes a set of putative direct mRNA targets, many of which were already associated with other AAGUGC-containing miRNAs, particularly those regulating tumor-suppressor genes. Altogether, our ongoing work underscores the critical role of newly emerged miRNA genes during evolution of placental mammals, opening new avenues for understanding how recently-evolved miRNA-mediated gene regulation may have shaped development and functions of the placenta in the primate lineage.

Comprehensive miR-21-binding site identification and mediated repression in hepatocytes

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microRNAs (miRNAs) are small RNA molecules that post-transcriptionally repress target RNAs, with each miRNA potentially regulating multiple targets and several miRNAs acting on a single RNA, resulting in complex synergistic effects. With over 2,600 miRNAs identified in the human genome, their interactions with RNAs form a finely-tuned regulatory network critical for gene expression control. A comprehensive understanding of miRNA-binding sites is essential to uncover their specific roles in cellular processes and the mechanisms of gene repression. In this study, we used miR-21 in human hepatocytes as a pathophysiological model due to its high expression in hepatocytes and its involvement in liver disease pathogenesis, including hepatocellular carcinoma. Using the iCLIP2 approach in both control and miR-21 knockdown conditions, we generated miRISC libraries by immunoprecipitating Ago. Our newly developed optiCLIP pipeline, designed to identify the most reproducible peaks across replicates, revealed 52,912 binding sites for the top 24 enriched miRNAs in our dataset. From these, we identified 578 miR-21-specific binding sites, corresponding to 411 unique targets. Further analysis of miR-21 binding site positions within the peaks indicated that central binding sites tend to be stronger than the peripheral ones. Accordingly, the majority of miR-21 binding sites were centrally located in the 411 direct target cohort compared to other miRNAs. By RNA-seq analysis in hepatocytes, we identified 75 direct target mRNAs significantly upregulated upon miR-21 knockdown. Finally, Ingenuity Pathway Analysis revealed that miR-21 directly regulates the PPAR signaling and JAK-STAT pathways, overall impacting on cholesterol metabolism in hepatocytes. In conclusion, we comprehensively studied the direct mechanism of miR-21 in hepatocytes to gain insight into the mode of action and the impact of miRNAs in cell physiology.

Analysis of microRNA and mRNA co-sequencing data at the single-cell level

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microRNAs (miRNAs) are small non-coding RNAs that play pivotal roles in the post-transcriptional regulation of gene expression, influencing various physiological and pathological processes, in particular in cancer initiation and development. In recent years, advancements in single-cell experimental techniques have revolutionized our understanding of cellular heterogeneity. Single-cell experiments enable to capture the dynamic of individual cells within a complex microenvironment and could contribute to the community's improved understanding of the role of microRNA in various biological processes. However, single-cell miRnome datasets are still relatively novel and scarce. An article published in 2019 (Wang et al. 2019), has conducted microRNA and mRNA co-sequencing for 19 K562 single-cells as a proof of concept. The article presents a short statistical analysis of correlations between microRNAs and their predicted mRNA targets. This type of dataset is exceptionally valuable for gaining a deeper understanding of how microRNAs affects the expression of their mRNA targets. Nevertheless, the analysis must be conducted using systematic approaches and with utmost care. We performed a thorough and comprehensive analysis of this dataset to comprehend both its potential and limitations. We have identified various parameters, such as target conservation through evolution or target expression, which can enhance the analysis. We have demonstrated that the original article's analysis was conducted with a bias, an issue we elucidate and rectify in our systemic analysis. To the best of our knowledge, there are only two other comparable datasets of miRNA- and mRNA co-sequencing at the single-cell level (Xiao et al. 2018 and Isakova et al. 2021), that we analyzed similarly, aiding in the validation of our initial findings.

Cryptic cytoplasmic long noncoding RNAs are pervasively translated in yeast and human cells

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Long non-coding (lnc)RNAs regulate multiple cellular processes. Although they were predicted to lack coding potential, recent works have revealed that some lncRNAs can be translated, resulting in the production of lncRNA-derived peptides. However, despite the interest they arouse, the potential of these peptides and the mechanisms controlling their synthesis have been poorly characterized. Here, we investigated the functional impact of non-canonical translation events on cytoplasmic lncRNAs in yeast and human cells. We show that Xrn1-sensitive cytoplasmic lncRNAs (XUTs) in yeast are mainly targeted by the Nonsense-Mediated mRNA Decay (NMD) pathway, indicating a translation-dependent degradation process. Ribo-seq confirmed ribosomes binding to XUTs and identified ribosome-associated 5'-proximal small ORFs. Mechanistically, the NMD-sensitivity of XUTs mainly depends on the 3'-UTR length. Moreover, we show that the peptide resulting from the translation of an NMD-sensitive XUT reporter exists in NMD-competent cells, suggesting that despite the cryptic nature of the transcript, its translation results in a detectable product. In human cells, we identified DIS3 as the main exonuclease restricting accumulation of lncRNAs in the cytoplasm and revealed thousands of DIS3-sensitive lncRNAs (DISTs). We show that DISTs also display active translation, producing peptides predicted to be high-affinity antigens in multiple myeloma patients carrying DIS3 mutations. Overall, our work highlights the central role of translation in the metabolism of cytoplasmic lncRNAs, with different potential outcomes. While the resulting peptides could constitute raw material exposed to the natural selection in yeast, we propose that some of them could be part of the cell-to-cell communication through tumor-specific antigen presentation in human cells.

HBZ-Related Dysregulation in Circular RNA Biogenesis during Adult T-Cell Leukemia Development

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Adult T-cell Leukemia/Lymphoma (ATL) is an aggressive malignancy of CD4+ T-cells caused by the Human T-cell Leukemia Virus type 1 (HTLV-1). ATL is marked by significant transcriptome reprogramming, including alterations in alternative splicing profiles of both protein-coding and non-coding RNAs. Circular RNAs (circRNAs), a class of non-coding RNAs forming covalently closed loops via back-splicing, have emerged as an important regulator of gene expression in cancer but remain unexplored in the context of ATL. This study investigates the biogenesis and functional relevance of circRNAs in ATL, focusing on the influence of the viral oncogene HBZ (HTLV-1 bZIP factor) on circRNA biogenesis mediated by RNA helicase such as DHX9. We conducted RNA-sequencing (RNA-seq) analyses on a cohort of 56 ATL patient samples across different disease stages (chronic, acute, and lymphomatous) and 8 healthy donor samples. Comparative analyses were performed using Jurkat cell lines whether or not expressing HBZ to characterize circRNA expression profiles. The role of the RNA helicase DHX9 in circRNA expression was examined through siRNA-mediated depletion, assessing its impact on back-splicing events upon HBZ expression. RNA-seq analyses revealed significant alterations in circRNA expression profiles in both ATL patient samples and HBZ-expressing Jurkat cells, identifying unique subsets of HBZ-deregulated circRNAs across different ATL stages. Notably, several HBZ-regulated circRNAs were identified, including circAFF2, which is potentially linked to tumor aggressiveness. These findings were validated using RNase R-based reverse transcription-quantitative PCR (RT-qPCR). Functionally, the in vitro production of circRNAs, particularly the delivery of synthetic circAFF2, significantly increased the proliferation of HBZ-expressing and HTLV-1-transformed cells. Conversely, inhibition of endogenous circAFF2 reduced tumor cell viability, underscoring the critical role of circRNA deregulation in ATL cell survival through the HBZ axis. At the molecular level, Co-IP assays demonstrated that HBZ interacts with DHX9, a key regulator of circRNA biogenesis. UV Cross-Linking and Immunoprecipitation (UV-CLIP) confirmed that DHX9 binds to pre-mRNAs producing HBZ-regulated circRNAs. Combined siRNA depletion and RNA Immunoprecipitation (RIP) analyses revealed that HBZ enhances the recruitment of DHX9 primary RNA while inhibiting its helicase activity. This inhibition leads to the accumulation of double-stranded RNA structures that promote the formation of circRNA and modulate back-splicing events controlled by the DHX9 complex. This study highlights the significant role of circRNAs as novel gene regulators in ATL, modulated by HBZ. The interaction between HBZ and the RNA helicase DHX9 leads to the deregulation of circRNA biogenesis, particularly affecting circAFF2, which contributes to tumor aggressiveness through cell proliferation.

A natural single nucleotide mutation in the small regulatory RNA ArcZ of the Plant pathogenic bacteria Dickeya solani switches off its antimicrobial activities against yeast and bacteria, and impact its virulence.

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The emergence of the necrotrophic plant pathogenic bacterium Dickeya solani in the European potato agrosystem has prompted extensive research into its genetic and pathogenic profile. All isolated strains of D. solani harbor several large polyketide synthase/non-ribosomal peptide synthetase (PKS/NRPS) gene clusters. Comparative analysis with analogous genes in other bacteria indicates that the clusters ooc and zms are responsible for the production of secondary metabolites from the oocydin and zeamine families, respectively. Additionally, a third cluster, designated sol, produces a novel antibiotic named solanimycin, which exhibits potent antifungal activity against various yeasts, including the human pathogen Candida albicans. In this study, we investigated the antimicrobial properties of these three PKS/NRPS clusters across different wild-type D. solani isolates. Through phenotyping and comparative genomics, we discovered that the small regulatory RNA ArcZ is crucial in regulating the sol and zms clusters. Notably, a single-point mutation, conserved in some wild-type strains of D. solani, including the type strain IPO 2222, disrupts ArcZ function by hindering its maturation into an active form. This mutation, which is also conserved across other Dickeya species, significantly impacts the virulence of D. solani in plants. Our findings demonstrate that single-nucleotide polymorphisms (SNPs) in sRNA-encoding genes can profoundly affect bacterial phenotypes. These insights underscore the importance of considering allele diversity in sRNA genes when studying bacterial pathogenicity and regulatory mechanisms. This research advances our understanding of bacterial regulation and pathogenesis, highlighting the intricate genetic factors that influence the behavior and impact of plant pathogens like Dickeya solani.

Ribosomal RNA synthesis by RNA polymerase I is regulated by premature termination of transcription

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The RNA polymerase I (Pol I) enzyme that synthesizes large rRNA precursors, exhibits high rate of pauses during elongation, indicative of a discontinuous process. We show here that Premature Termination of Transcription (PTT) by Pol I is a critical regulatory step limiting rRNA production in vivo. The Pol I mutant, SuperPol (RPA135-F301S), produces 1.5-fold more rRNA than the wild type (WT). Combined CRAC and rRNA analysis link increased rRNA production in SuperPol to reduced PTT, resulting in shifting polymerase distribution toward the 3' end of rDNA genes. In vitro, SuperPol shows reduced nascent transcript cleavage, associated with more efficient transcript elongation after pauses. Notably, SuperPol is resistant to BMH-21, a drug impairing Pol I elongation and inducing proteasome-mediated degradation of Pol I subunits. Compared to WT, SuperPol maintains subunit stability and sustains high transcription levels upon BMH-21 treatment. These comparative results show that PTT is alleviated in SuperPol while it is stimulated by BMH-21 in WT Pol I.

RNase W, a conserved ribonuclease family with a novel active site

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Ribosome biogenesis is a complex process requiring multiple pre-rRNA cleavage steps. In archaea, the full set of ribonucleases involved in rRNA processing remains to be discovered. A previous study suggested that FAU-1, a conserved protein containing an RNase G/E-like protein domain fused to a domain of unknown function (DUF402) acts as a ribonuclease in archaea. However, the molecular basis of this activity remained so far elusive. In our results, we report two X-ray crystallographic structures of RNase G/E-like-DUF402 hybrid proteins from Pyrococcus furiosus and Sulfolobus acidocaldarius, at 2.1 A and 2.0 A, respectively. The structures highlight a structural homology with the 5' RNA recognition domain of Escherichia coli RNase E but no homology with other known catalytic nuclease domains. Surprisingly, we demonstrate that the C-terminal domain of this hybrid protein, annotated as a putative diphosphatase domain, harbors the ribonuclease activity. Our functional anal- ysis also supports a model by which the RNase-G/E-like domain acts as a regulatory subunit of the ribonuclease activity. Finally, in vivo experiments in Haloferax volcanii suggest that this ribonuclease participates in the maturation of pre-16S rRNA. Together our study defines a new ribonuclease family, that we termed the RNase W family, as the first archaea-specific contributor to archaeal ribosome biogenesis.

Cytoplasmic uridylation monitors maturation of the 18S rRNA 3' end

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Diamond-Blackfan anemia (DBA) is a ribosomopathy linked to the haploinsufficiency of more than 20 ribosomal protein genes. A significant fraction of these proteins are required for the proper maturation of the 3' end of the 18S ribosomal RNA (rRNA). In mammalian pre-40S particles, formation of the 18S rRNA 3' end involves 3'-5' exonucleolytic trimming of around 80 nucleotides from the 18S-E pre-rRNA, followed by a final endonucleolytic cleavage by NOB1. This trimming process is catalyzed in part by the exoribonuclease PARN and occurs concomitantly with the nuclear export of pre-40S particles. Using high-throughput 3' RACE-seq, we have established the complete nucleotide-resolution landscape of this exonucleolytic process in human cells. Our findings reveal that 3' adenylation and uridylation are integral to the 18S-E pre-rRNA maturation process. Adenylation modifies the 3' end at nearly every position during exonucleolytic trimming, whereas uridylation is largely restricted to nucleotides forming a central stem-loop in this region. Importantly, 3' uridylation is restricted to the cytoplasm and is mediated by the terminal uridylyl transferases TUT4 and TUT7. Knockdown of TUT4 and TUT7 delays both stem-loop processing and the protein remodeling in pre-40S particles. This phenotype is further exacerbated when pre-40S particle maturation is challenged by knockout of small ribosomal subunit proteins (RPSs). Under these conditions, the surveillance pathway mediated by the exoribonuclease DIS3L2 is activated, recruiting DIS3L2 to long, uridylated 3' extensions. Consistent with a quality-control role, we observed that RPS-haploinsufficient lymphoblastoid cells from Diamond-Blackfan anemia patients display higher rates of 18S-E pre-rRNA uridylation. We propose that 3' uridylation plays a dual role: it contributes to pre-40S particle maturation under normal conditions and activates a cytoplasmic quality-control pathway when this process is impaired. This first high-resolution overview of 18S rRNA 3' end processing in humans uncovers a novel role for 3' uridylation in the cytoplasmic control of 18S-E pre-rRNA maturation, particularly in managing a strong secondary structure, under both steady-state and stress conditions.

Cryo-EM Structures of Saccharolobus solfataricus Initiation Complexes with Leaderless mRNAs

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The archaeal ribosome is of the eukaryotic type. Genomic and phylogenetic studies have indicated that TACK and Asgard, the closest relatives of eukaryotes, have ribosomes containing eukaryotic ribosomal proteins not found in other archaeal branches, eS25, eS26 and eS30. In our study, we investigated the case of Saccharolobus solfataricus, a crenarchaeon belonging to the TACK branch, which mainly uses leaderless mRNAs. We characterized the small ribosomal subunit of S. solfataricus bound to SD-leadered or leaderless mRNAs (lmRNAs). Cryo-EM structures show for the first time archaeal versions of eS25, eS26 and eS30 proteins bound to the small subunit. In addition, we identify two novel ribosomal proteins named aS33 and aS34 as well as a domain of eS6, that highlight the diversity of archaeal ribosomes. Leaderless mRNAs are bound to the small ribosomal subunit, and the 5'-triphosphate group contributes to their binding. Archaeal eS26 is in the mRNA exit channel wrapped around the 3' end of ribosomal RNA, as it is in eukaryotes. Its position is not compatible with an SD:antiSD duplex in the mRNA exit channel. Overall, our results suggest a role of eS26 in translation regulation and possible evolutionary routes from archaeal to eukaryotic translation.

A comprehensive, multi-omics and structure-function study of mutant 'onco-ribosomes' involved in Chronic Lymphocytic Leukemia.

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Control of gene expression is at the heart of cellular fate, and ribosomes, the universal ribonucleoproteic machines in charge of translating mRNAs into proteins, are key players of gene expression regulation. Ribosomes have long been thought to harbor strictly identical composition and production capacities for each cell of an organism. However, the heterogeneity of ribosomes has been highlighted by the discovery of mutations in numerous ribosomal proteins (RP) associated with different cancer types [1]. Missense point mutation of RPL10 (uL16) have been shown to be drivers of T-cell Acute Lymphoid Leukemia (T-ALL). These mutations alter the affinity for mRNA translation regulatory sequences, resulting among others in the hypertranslation of oncoproteins as JAK-STAT signaling components or anti-apoptotic protein BCL2 [2,3]. Therefore, mutations in ribosomal proteins of this essential apparatus are associated with various cancers, but whether and how other RP mutations can rewire translation to lead to oncogenic mechanisms remains elusive. Our study focuses on RPS15 (uS19), a protein belonging to the small ribosomal subunit, whose point mutations in the C-terminus domain are found in up to 20% patients suffering from Chronic Lymphoid Leukemia (CLL) [4]. As this domain is located in the active site of ribosomes, its mutations might directly affect translation steps (such as initiation, elongation...), and consequently alter the translatome of cells displaying them. To characterize their molecular and cellular effect, we generated an ‘onco-ribosome' cell line library, (ie CRISPR-Cas9 engineered isogenic lymphoid Ba/F3 cells) expressing homozygous mutant ribosomal proteins, among which P131S and H137 point mutations in RPS15. Genomewide translatome analysis of the onco-ribosome library revealed a significant translational rewiring in RPS15-mutant cells, as well as their altered codon usage. Using cryo-electron microscopy, we performed a comparison of the 3D structures of both WT and onco-ribosomes, which showed significant differences in the dynamics of their translation elongation cycle. These structural observations were further reinforced by biochemical assays and cell-growth analysis using site-specific ribosomal antibiotics.
Altogether, our comprehensive study of RPS15 onco-ribosomes suggest that mutations in RPS15 C-terminal domain have a direct impact on the translation elongation mechanics, which will particularly affect the expression of transcription and autophagy regulators, and in turn the cell's proliferation capacities. References: [1] Kampen, K. R., et al. Nucleic Acids Res. (2019). [2] Girardi, T. et al. Leukemia 32, 809–819 (2018). [3] Kampen, K. R. et al. Leukemia 33, 319–332 (2019). [4] Ljungstrom, V. et al. Blood 127, 1007–1016 (2016).

Intricate ribosome composition and translational reprogramming in epithelial-mesenchymal transition

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Epithelial-mesenchymal transition (EMT) involves profound changes in cell morphology, driven by transcriptional and epigenetic reprogramming.a EMT programs exhibit great diversity, based primarily on the distinct molecular activities of the EMT transcription factors, which need to be thoroughly characterized.a However, evidence suggests that translation and ribosome composition also play key roles in establishing pathophysiological phenotypes. Using genome-wide analyses, we reported significant rearrangement of the translational landscape and machinery during EMT. Specifically, a mesenchymal cell line overexpressing the EMT transcription factor ZEB1 displayed alterations in translational reprogramming and fidelity. Furthermore, using RiboMethSeq and riboproteomics we unveiled a decreased level of rRNA 2'Ome of 28S_Um2402 as well as an increased level of the ribosomal protein RPL36A in mesenchymal ribosomes, indicating precise tuning of ribosome composition. Remarkably, RPL36A overexpression alone was sufficient to trigger the acquisition of mesenchymal features, demonstrating its pivotal role in EMT. These findings underline the importance of translational reprogramming and fine-tuning of ribosome composition in EMT.

Hijacking of the RNA helicase, UPF1 reveals its proviral function during HTLV-1 infection.

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In the past, we showed that during HTLV-1 and HIV-1 infections, the RNA decay pathway called NMD (Nonsense mediated MRNA Decay), plays an antiviral function, notably by targeting the unspliced viral RNA (gRNA). To protect gRNA, we characterized how the proteins Rex and Rev (from HTLV and HIV) sequester the NMD helicase UPF1 in the nucleus, in a CRM1 dependant manner, leading to NMD inhibition. Alongside, CRM1 is an exportin demonstrated to be hijacked by Rex/Rev to export gRNA: Rex/Rev bind simultaneously to CRM1 and gRNA, while CRM1 drives the nuclear export of this viral RNP. Due to the common involvement of Rex and CRM1, we wonder if there is a coordination between NMD inhibition and gRNA export. To address these matters, we performed protein immunoprecipitations in different HTLV-1 infected lymphocytes, expressing Rex or not. We found that the UPF1/CRM1 complex is strongly favoured/stabilized in the presence of Rex. To study its consequences for UPF1, we modelled UPF1/CRM1 interaction: first we precisely identified the NES domain of UPF1 before performing molecular dynamic simulations with coarse grain representation. These experiments suggest that when associated with CRM1, UPF1 may have a reduced affinity for RNA, what matches with the observed NMD inhibition. To challenge these simulations more precisely, we performed RNA IP that corroborated a lower binding of UPF1 with endogenous RNA, when Rex favours UPF1/CRM1 binding. To the contrary, in the presence of Rex, UPF1 was more loaded on vRNA, driven by the specific binding of Rex to the RxRE motif of vRNA. In this context, luciferase assays and fractionations showed that UPF1 was indeed needed to favour nuclear export of HTLV-1 vRNA. In the late steps of retroviruses replication cycle, vRNA is encapsidated in viral particles. Combining confocal microscopy, mass spectrometry and RNA IP, we also demonstrated that UPF1 is also incorporated in HTLV-1 virions, associated with vRNA. However, after UPF1 extinction, vRNA incorporation in viral particles, as well as GAG maturation, are significantly reduced, independently from NMD inhibition. Finally, by monitoring viral transmission between C91PL and uninfected Jurkat, we could correlate these deregulations with defects in virions infectiousness. Altogether, our data demonstrate that NMD inhibition by Rex is coordinated with UPF1 hijacking and specific redirection towards gRNA. As a part of the vRNP, UPF1 supports viral functions during HTLV-1 replication cycle, ultimately favouring the viral transmission.

Identification of a bifunctional RNA involved in the virulence of Dickeya dadantii

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Non-coding RNAs play an important role in the regulation of gene expression in a variety range of organisms. In addition to antisense anda trans-acting RNAs, which are well described in bacteria, other regulatory mechanisms have been proposed. Some sRNAs have been found to encode peptides, and long transcripts have been found to repress the expression of the overlapping transcripts produced by neighboring genes. This suggests that RNA plays an important role in regulatory networks. We deciphered the transcriptional landscape of the plant pathogena Dickeya dadantii, whicha causes soft rot disease in a wide range of plant species, including economically important crops such as potato and rice. During our analysis, we identified that several virulence regulator mRNAs overlapped with mRNAs of neighboring genes. The neighboring genes were predicted to encode proteins involved in either metabolism or the stress response. However, their role in bacterial-host interactions remains unclear. The present study focuses on thea pecSa mRNA, which encodes a transcriptional regulator described to repress the premature expression of virulence factors leading to maceration of the plant tissue. The transcriptional landscape ofa D. dadantiia revealed that thea pecSa mRNA overlaps with the mRNA of the convergent genea argGa at the common terminator region. We hypothesize that the two transcripts may regulate each other through mRNA-mRNA interaction. Sincea argGa is expressed in the absence of arginine and PecS represses virulence factors, the level of arginine may affect the virulence ofa D. dadantii. Our results show that the amount of the PecS protein is significantly decreased in the absence of arginine and that this is not due to a regulation of transcription initiation. To assess whether increased production of one transcript can titrate the convergent transcript, we overexpressed one of the transcripts and analyzed the effect on the other transcript by quantifying the protein level. The results suggest that thea pecSa anda argGa transcripts are bifunctional RNAs that play the role of both mRNAs and antisense RNAs. Finally,a in plantaa experiments are also performed to evaluate the effect of apoplastic arginine on the infection process ofa D. dadantii.

Deciphering the impact of SARS-CoV-2 infection on cellular RNA machineries localization and function

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RNA viruses are a major cause of emerging infectious diseases. Recently, a series of variants of concern with immune escape adaptations rapidly emerged from an ancestral SARS-CoV-2 strain, highlighting the need for robust therapeutic targets. Like other RNA viruses, SARS-CoV-2 relies on the host's RNA machinery to express and replicate its genome while evading immune detection. Several studies have focused on determining the repertoire of physical interactions between SARS-CoV-2 RNA or proteins and host RNA-binding proteins (RBPs); however, the effect of infection on RBP subcellular localization remains unclear. Furthermore, it is unknown whether changes in RBP localization are part of the virus's adaptation. We therefore utilized a collection of experimentally validated antibodies targeting more than 200 RBPs to assess their subcellular localization during infection using high-content microscopy. Four different variants of concern were compared. Changes in RBP localization were correlated with the transcriptome and proteome of infected cells. The results indicate perturbations in the assembly of various ribonucleoprotein particles, which correlate with transcript processing defects, with both similarities and differences across variants. We also noted significant differences in host and viral RNA expression among the variants. By systematically evaluating the effects of these viral variants, this study aims to identify conserved targets of RNA virus infections to enhance pandemic preparedness.

SL RNA recognition by the unusual trypanosomatid cap-binding complex

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The RNA 5'-cap-binding complex is central to the fate of eukaryotic RNAs. Trypanosomatids are eukaryotic protozoans that include human parasitic pathogens like trypanosomes and Leishmania species, bearing unusual RNA processing pathways. The processing of their primary expressed pre-mRNA polycistrons requires the coupled action of trans-splicing and polyadenylation to yield monogenic mature mRNAs for all genes. As a result of the process, all mature mRNAs carry a unique and identical sequence, the SL RNA mini-exon with a hypermethylated cap-4 structure at their 5'-ends. The SL RNA is bound by the unusual trypanosomatid cap-binding complex (CBC). CBC is vital for the cell and necessary all mRNA processing. In difference to mammals and yeast, trypanosomatid CBC consists of four subunits, three of which lack sequence homology to annotated proteins.

Here, we present the structural and functional characterization of the Trypanosoma brucei CBC. We reveal the function of its subunits. Through binding assays with fully modified synthetic cap-4 SL RNA, we define the molecular SL RNA binding properties of trypanosomatid CBC. Our refined understanding of trypanosomatid CBC will now enable further detailed studies of RNA processing pathways in these parasites. Due to the non-conserved nature of the complex, it may serve as a future anti-infective drug target.

Modulation of antiviral innate immunity by the human Dicer protein

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In mammals, the co-existence of RNAi and the type I interferon response in somatic cells begs the question of their compatibility and relative contribution during viral infection. Previous studies provided hints that both mitigating co-factors and self-limiting properties of key proteins such as Dicer could explain the apparent inefficiency of antiviral RNAi. Indeed, the helicase domain of human Dicer limits its processing activity and acts as an interaction platform for co-factors that could hinder its function. We studied the involvement of several helicase-truncated mutants of human Dicer in the antiviral response. We show that all deletion mutants display an antiviral phenotype against alphaviruses and an enterovirus. While only one of them, Dicer N1, is antiviral in an RNAi-independent manner, they all require the expression of PKR to be active. To elucidate the mechanism underlying the antiviral phenotype of Dicer N1 expressing cells, we analyzed their transcriptome and found that many genes from the interferon and inflammatory response were upregulated. We could show that these genes appear to be controlled by transcription factors such as STAT-1, STAT-2, and NF-kB. Finally, we demonstrated that blocking the NF-kB pathway in Dicer N1 cells abrogated their antiviral phenotype. Our findings highlight the crosstalk between Dicer, PKR, and the IFN-I pathway, and suggest that human Dicer may have repurposed its helicase domain to prevent basal activation of antiviral and inflammatory pathways.

POSTER INFORMATION & ABSTRACTS

- Posters can be hung up as soon as you arrive
- Posters should be removed by November 19 at 6 p.m. at the latest.
- Poster exhibition will take place in the Espace Mérieux and in the Salle Condorcet.

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The oncoprotein MYCN antagonizes the effect of helicases DDX17 and DDX5 and stimulates the production of readthrough-induced chimeric transcripts

Clerc Valentine [1], Valat Jessica [1], ***Aouadi Khouaila [1],*** Grand Xavier [1], Fontrodona Nicolas [1], Bazire Mateo [1], Rama Nicolas [2], Gibert Benjamin [2], Mortreux Franck [1], Bourgeois Cyril [1]

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We have previously shown that DEAD box helicases DDX17 and DDX5 control the correct termination of transcription and the associated cleavage of the 3' end of transcripts (Terrone et al., 2022). Here we show that the transcriptional readthrough induced by their depletion in neuroblastoma cells also results in increased production of chimeric transcripts from tandemly oriented genes. Analysis of neuroblastoma tumours, in which chimeric transcripts are abundant, revealed that low expression of DDX17 and DDX5 is significantly associated with poor patient survival and high-risk tumours, and inversely correlated with MYCN oncogene amplification. Surprisingly, MYCN does not affect the expression of either helicase, but it does alter transcription termination and chimeric transcript production. We provide evidence that MYCN directly promotes transcription readthrough by binding to the 3' region of genes and that it interacts with DDX17, suggesting that it could alter the activity of the helicase, although the exact mechanism is still unclear. Our work reveals a novel function of MYCN in transcription termination and suggests that the combined deregulation of MYCN and DDX17 expression may lead to readthrough-associated transcriptome changes in neuroblastoma. We are also investigating the functional consequences of chimeric transcript expression in cancer cells.

Triplex Decoy Oligonucleotides as Modulators of Long Non-coding RNA Activities

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Gene expression dysregulation is a major factor in numerous human diseases, including cancers, cardiovascular, and neurodegenerative disorders. Restoring proper gene regulation is critical, but it poses challenges, especially regarding the specificity of therapeutic agents. Long non-coding RNAs (lncRNAs), non-protein-coding transcripts longer than 200 nucleotides, have emerged as key regulators of gene expression, acting in both the cytoplasm and the nucleus. Nuclear lncRNAs can associate with the genome to recruit epigenetic complexes, which in turn mediate chromatin remodeling and influence gene expression. Thus, dysregulation of lncRNAs can affect these processes, promoting diseases like cancer by modulating cell proliferation, apoptosis, and differentiation for instance. One well-known lncRNA, ANRIL (Antisense Noncoding RNA in the INK4 Locus), transcribed from the 9p21 region, regulates gene expression both in cis and trans. In cis, ANRIL silences tumor suppressor genes CDKN2A and CDKN2B, which control cell cycle progression. In trans, ANRIL regulates genes involved in inflammation, chromatin organization, and cell growth across the genome. Previous studies by the team demonstrated that ANRIL binds to thousands of genomic loci in humans, with Exon 8 playing a crucial role in this association. This interaction is believed to occur, at least in part, through triplex formation, where a single-stranded RNA accommodates the major groove of double-stranded DNA via Hoogsteen hydrogen bonds. Overexpression of Exon8-containing ANRIL isoforms has been associated with several pathologies, including coronary artery disease, diabetes, and a wide range of cancers, such as gastric, prostate, and brain cancers. To counteract the pathogenic effects of ANRIL, we develop a novel therapeutic strategy based on the use of Triplex Decoy Oligodeoxynucleotides (TDOs). TDOs are short DNA sequences designed to mimic the triplex-binding sites of ANRIL on the genome, thereby competing with genomic DNA for ANRIL association. By preventing ANRIL from forming triplexes with its target genes, TDOs are expected to neutralize its regulatory functions, potentially at least partially restoring normal gene expression and promoting normal cell proliferation. Evidences from in vitro experiments and mouse xenograft models suggest that TDOs are likely to be effective in this role. This approach shows potential for developing new therapies targeting lncRNA-mediated gene dysregulation, particularly in cancers where ANRIL overexpression is implicated.

Exon-junction complex association with stalled ribosomes and slow translation-independent disassembly

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Exon junction complexes are deposited at exon-exon junctions during splicing. They are primarily known to activate non-sense mediated degradation of transcripts harbouring premature stop codons before the last intron. According to a popular model, exon-junction complexes accompany mRNAs to the cytoplasm where the first translating ribosome pushes them out. However, they are also removed by uncharacterized, translation-independent mechanisms. Little is known about kinetic and transcript specificity of these processes. Here we tag core subunits of exon-junction complexes with complementary split nanoluciferase fragments to obtain sensitive and quantitative assays for complex formation. Unexpectedly, exon-junction complexes form large stable mRNPs containing stalled ribosomes. Complex assembly and disassembly rates are determined after an arrest in transcription and/or translation. 85% of newly deposited exon-junction complexes are disassembled by a translation-dependent mechanism. However as this process is much faster than the translation-independent one, only 30% of the exon-junction complexes present in cells at steady state require translation for disassembly. Deep RNA sequencing shows a bias of exon-junction complex bound transcripts towards microtubule and centrosome coding ones and demonstrate that the lifetimes of exon-junction complexes are transcript-specific. This study provides a dynamic vision of exon-junction complexes and uncovers their unexpected stable association with ribosomes.

A strategy to engineer short bursts of RNase H1 in live human cells to manipulate RNA:DNA hybrids

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DNA replication stress (RS) is believed to underlie the development of precancerous lesions. Strikingly, the hallmarks of DNA replication stress can be largely alleviated by the lengthy over-expression of RNase H1, a highly conserved enzyme that degrades the RNA moiety of RNA:DNA hybrids. This suggests that DNA replication stress somehow disrupts the metabolism of RNA:DNA hybrids in a way that is detrimental for genome integrity and stability. The origin and the identity of such stress-induced RNA:DNA hybrids has not yet been conclusively identified. How such stress-induced RNA:DNA hybrids interfere with genome stability is also still debated. To tackle these questions, it would be useful to manipulate the levels of RNA:DNA hybrids very quickly in live cells. To quickly map and manipulate RNA:DNA hybrids, we use Virus-Like Particles (VLPs) to deliver a panel of ready-made regulators and sensors of RNA:DNA hybrids directly into live cells. Our innovative strategy allows the delivery of physiologically-relevant levels of those proteins in the nuclei of human cells in culture in only three hours, which is compatible with the manipulation of RNA:DNA hybrid levels in specific phases of the cell-cycle. Here, I will present results obtained with the delivery of E.coli RNase H1 (RnhA) into live human cells. I will provide evidence that the VLP-delivered RnhA associates (i) with endogenous RNA:DNA hybrids and (ii) with replication forks. I will also show that, when delivered specifically during DNA replication, the VLP-delivered RnhA is able to modulate both the progression of replication forks in response to small doses of Hydroxyurea and the occurrence of Transcription-Replication Conflicts (TRCs), as monitored by the proximity of PCNA and RNA polymerase II. I will discuss the advantages of our approach to pinpoint the direct consequences of the loss of RNA:DNA hybrids on transcription and replication.

Towards the discovery of new riboswitches by very large-scale enzymatic screening

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Riboswitches are RNA structural motifs present in the 5'UTR of genes. Through interactions with a specific physical or chemical signal (temperature, metabolites, etc.), riboswitches modulate their structures between an on/off configuration that subsequently controls the regulation of the adjacent gene's expression. Regulation mechanisms can be transcriptional, translational, or post-transcriptional via the degradation of mRNAs [1]. Riboswitches offer important insights on bacterial stress responses, pathogenicity, and evolutionary origins via the “RNA world” Hypothesis. Additionally, the discovery and utilization of novel riboswitches in gene expression systems may have beneficial therapeutic (gene therapy), biotechnological (reporter assays), and/or industrial applications (cell factories). At present, there are ~60 unique, experimentally validated riboswitch classes, but an estimated 28,000 classes remain undiscovered in nature [2]. However, new riboswitch classes are difficult to find due their low abundance in nature. Bioinformatics approaches are limited in scope due to their reliance on fully annotated genomes, as well as the structural homology of new RNA motifs with previously known riboswitch classes. Traditional SELEX approaches do not effectively screen RNA motifs for structural changes following ligand binding that are characteristic of riboswitches. New methodologies are required to discover the full range of riboswitches and their corresponding effector signals. Recently, our team developed Helicase SELEX, an activity-focused method for riboswitch discovery based on iterative enzymatic screening (helicase activity) in the presence/absence of a signal molecule of interest that, in part, overcomes these methodological constraints. This method has discovered new natural riboswitches in E. coli and developed synthetic specimens controlled by a novel chemical signal (serotonin) [3, 4]. My PhD work focuses on adapting this proprietary method for large-scale riboswitch discovery, utilizing environmental metagenomes and complex molecular mixtures to maximize the rate and diversity of riboswitch discovery and the chemical signals that modulate their activity. The most promising candidates will be further characterized using biochemical and structural techniques in vitro and in vivo. Full automation of this process in addition to sophisticated bioinformatics will ideally offer an efficient method for finding new riboswitch candidates on a massive scale. References [1] Breaker, R.R. (2022). The biochemical landscape of riboswitch ligands. Biochemistry, 61(3), 137-149. [2] Kavita, K., Ronald, R.R. (2022). Discovering riboswitches: the past and the future. Trends in Biochemical Sciences, 48(2), 119-141. [3] Delaleau M., Eveno, E., Simon, I., Schwartz, A., Boudvillain, M. (2022) A scalable framework for the discovery of functional helicase substrates and helicase-driven regulatory switches. PNAS, 119(38), e2209608119. [4] Delaleau, M., Figueroa-Bossi, N., Do, T.D., Kerboriou, P., Eveno, E., Bossi, L. and Boudvillain, M. (2024). Rho-dependent transcriptional switches regulate the bacterial response to cold shock. Molecular Cell.

High-throughput identification and characterization of proteins interacting with retrotransposon RNA

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Retrotransposons are ubiquitous mobile genetic elements that can compromise the expression and stability of the host genome through insertional mutagenesis or ectopic recombination between their dispersed copies. While the biological importance of retrotransposons has long been underestimated, it is now well-established that they contribute to genome architecture and evolution. Yet in mammals, where they are generally repressed, their transcription reactivation is a feature of malignant cells and aging, and is associated with genome instability. In the retrotransposon replication cycle, RNA is a key intermediate as it serves as template for both translation and reverse-transcription. Retrotransposon RNA could also participate in genome instability, for instance by forming R-loops. Therefore, proteins that bind this RNA could be pivotal in retrotransposon replication and genome instability. Yet, we know little about retrotransposon RNA regulation by RNA-binding proteins. To address this question, we have set up the ChIRP-MS proteomic approach in yeast to identify the repertoire of proteins binding the RNA of Ty1, a retrotransposon model in S. cerevisiae. Based on stringent criteria, we selected a total of 29 candidates associated with Ty1 RNA, 66% of which had not yet been identified as regulators of retrotransposition. To shed light on the function of these Ty1 RNA-binding candidates, our current working plan is to i) validate and characterize the RNA-protein interactions; ii) study the candidates' impact on the different stages of retrotransposition cycle; iii) investigate their biological role on genome instability.

The R2TP chaperone mediates co-translational channeling of quaternary structure formation

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Many cellular mechanisms require multi-subunit machineries composed of proteins and RNAs. The correct assembly of the subunits is crucial to form functional complexes. However, in many cases, the timing and mechanism of assembly remains unclear, in particular with regards to the translation of the protein subunits. The HSP90/R2TP is a quaternary chaperone that assists the assembly of various multi-subunit machineries, including the three RNA polymerases and several non-coding RNPs, such as snoRNPs, snRNPs and miRNPs. The R2TP chaperone is composed of RUVBL1/2, RPAP3 and PIH1D1, and the details of its mechanism of action remain unclear. We now show by RIP-seq and single molecule imaging that R2TP binds many of the mRNAs coding for its client proteins. This occurs in a translation-dependent manner, indicating that it does not directly bind RNA but that it associates to its client co-translationally. Comparison of the R2TP partners found by RIP-seq and quantitative proteomics reveals three groups of proteins partners: the ones binding R2TP both co- and post-translationally, the ones binding only post-translationally and, surprisingly, some that bind only co-translationally. This latter category reveals many new R2TP partners, suggesting an unexplored role of this quaternary chaperone during co-translational events. Indeed, inhibition of R2TP leads to the formation of POLR2A mRNA condensates. These condensates are driven by the nascent protein, suggesting that R2TP escorts clients from synthesis to assembly to prevent unwanted reactions. Multicolor smFISH for pairs of subunits belonging to the same complex shows that only few mRNAs colocalize (1-3%). This indicates that client subunits are only rarely co-translated and co-assembled, and are rather translated separately and assembled together later. Altogether, these data suggest that the R2TP chaperone channels subunits from their translation to their assembly and that this could be a widespread mechanism.

Translation initiation in Saccharolobus solfataricus

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Archaeal translation initiation has both bacterial and eukaryotic features. Archaeal initiation factors, aIF1, aIF1A, aIF2, and aIF5B correspond to a subset of eukaryotic translation initiation factors. In contrast, archaeal mRNAs are not processed after transcription and are therefore of the bacterial type. Transcriptomic analyses have shown that depending on the archaeal phylum, mRNAs mainly contain Shine-Dalgarno (SD) sequences or have very short (≤ 7 nucleotides) or no 5_-untranslated regions (UTR). mRNAs belonging to the latter class are called leaderless. The whole data suggest that leaderless mRNAs and leadered mRNAs with SD sequences co-exist in all archaea. In some archaea, most mRNAs are leaderless, for instance Haloferax volcanii (72%) or Saccharolobus solfataricus (69 %). We studied 30S translation initiation complexes from Saccharolobus solfataricusprepared with mRNAs having different 5'-untranslated regions. Toeprinting experiments and cryo-EM structures identified unexpected specificities of the small crenarchaeotal subunit that illustrate the evolutionary diversity in archaea. Our work shows for the first time archaeal versions of the ribosomal proteins eS30, eS25 and eS26. Sequence specificities of archaeal eS30 and eS25 are discussed in the light of their role in translation. We have also studied leaderless mRNAs binding and shown that the 5'-triphosphate group contributed to leaderless mRNA binding through a network of water molecules that mimics a mRNA base located at position -1. Finally, we showed that the binding of eS26 in the mRNA exit channel is not compatible with the SD:antiSD duplex. This suggests that eS26 could regulate leaderless versus leadered mRNA translations. Collectively, our study is part of the current trend towards comparative studies that provide a better understanding of the evolution of the translation machinery.

rRNAs: stable or instable?

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Abstract: RNA decay is a complex enzymatic process with thousands of substrates binding to the same enzymes in the degradation machinery. How the different RNA substrates compete in vivo for this machinery is still a mystery. We investigate the substrate/enzyme competition mechanism when RNAs compete for the enzyme that initiates their decay process. This uncharacterized mechanism of RNA decay competition is explored in the bacterial model, Escherichia coli, where RNase E is responsible for the initial internal cleavage of RNAs1. Previous works have suggested that RNase E is limiting in E. coli cells2 and that the competition between cellular messenger RNAs (mRNAs) for RNase E binding could play a role in the regulation of mRNA half-lives2,3. It has also been shown that RNase E contributes to the destabilization of ribosomal RNAs (rRNAs) under conditions when these abundant “stable” RNAs are no longer as "stable" as is usually supposed4,5. These new findings raise a question on the RNA competition mechanism: are rRNA also involved in the competition for RNase E binding similarly to mRNAs? Methods: We studied rRNA stability with an E. coli K12 strain growing at OD600 = 0.5 on LB. RNA synthesis was inhibited with rifampicin and samples were taken at different times after rifampicin addition (15, 30 and 60 minutes). Samples were quenched with 5% phenol (in ethanol), centrifuged and stored at -20°C. Two methods of cell disruption were compared: glass beads versus Multi-therm. Total RNA was then extracted using different RNA extraction protocols. RNA was fractionated on agarose gels and quantified using Image Lab. Results: When the cells are broken with the Multi-therm, a loss of 23S and 16S rRNAs of 83% and 91% respectively is observed after one hour, while 5S rRNA remains stable. These results are in perfect agreement with those reported in the literature using the same procedure4,5. In contrast, when the cells are broken with glass beads, no loss of 23S and 16S rRNAs is measured (the 5S rRNA also remains stable). In an attempt to understand the loss of 23S and 16S rRNAs with the Multi-therm protocol, we performed different tests during sampling and RNA extraction: measurement of genomic DNA concentration, DNase treatments, Proteinase K treatments, addition of a centrifugation step... The results allowed us to look at possible explanations. Conclusion: We show that, depending on the cell breaking method, the profiles of 23S and 16S rRNA concentrations after rifampicin addition are not the same. This result raises the question of the real biological stability of rRNAs. Our conclusion is that rRNAs are truly stable and the loss of 23S and 16S rRNAs observed over time with Multi-therm is only an artefact of the protocol. Therefore, rRNAs should not be included in the competition between RNAs for RNase E degradation. 1. Strahl, H, et al. (2015) Membrane recognition and dynamics of the RNA degradosome. PLoS Genet, 11:e1004961. 2. Etienne, TA, et al. (2020) Competitive effects in bacterial mRNA decay. J Theor Biol:110333. 3. Nouaille, S, et al. (2017) The stability of an mRNA is influenced by its concentration: a potential physical mechanism to regulate gene expression. Nucleic Acids Res, 45:11711-11724. 4. Hadjeras, L, et al. (2023) Attachment of the RNA degradosome to the bacterial inner cytoplasmic membrane prevents wasteful degradation of rRNA in ribosome assembly intermediates. PLoS Biol, 21:e3001942. 5. Hamouche, L, et al. (2021) Ribosomal RNA degradation induced by the bacterial RNA polymerase inhibitor rifampicin. RNA, 27:946-58.

Epitranscriptomic modification of the mRNA cap and study of its translation mechanism

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Epigenetic modifications of RNA play a crucial role in regulating gene expression. The canonical cap-dependent translation of mRNAs relies on the recognition of a 7-methylguanosine (m7G) cap at the 5' end of the mRNA by the initiation factor eIF4E, a necessary step for ribosome recruitment. Our laboratory was the first to identify an epigenetic cap modification in mammals, revealing that stress-related selenoprotein mRNAs possess a hypermodified 2,2,7-trimethylguanosine (TMG) cap (1,2). This hypermethylation is catalyzed by trimethylguanosine synthase 1 (Tgs1). Notably, these mRNAs are not recognized by eIF4E, yet they undergo translation via an unknown mechanism (3). Using TMG cap immunoprecipitation and RNA sequencing (TMG RIP-seq), we have uncovered the full repertoire of TMG-capped mRNAs, identifying 349 novel TMG-capped mRNAs, involved in translation and stress responses.

To further investigate the localization and translation of a subset of these mRNAs (including COX7C, TMBIM6, PDE6D, RPL32, MRPL53, WDR59, and SPC24), we performed subcellular and polysome fractionation followed by TMG cap immunoprecipitation in HEK293FT cells. Our findings reveal that TMG-capped mRNAs are predominantly located in the cytoplasm, are associated with polysomes and thus translated. TMG-capped mRNAs can furthermore be translated in vitro in cell free extracts. Ongoing efforts aim to further characterize the ratio of endogenous TMG to m7G-capped mRNAs using mass spectrometry.

These findings suggest a potential novel translation mechanism mediated by hypermethylation of the m7G cap, expanding possibilities for understanding RNA regulation. 1. Wurth L. et al. (2014) Nucleic Acids Research, 42, 8663-867. 2. Gribling-Burrer A.S. et al. (2017) Nucleic Acids Research, 45, 5399-5413. 3. Hayek H. et al. (2022) Biomolecules, 9, 1268.

Association of RNA-degrading machines with the ribosome in archaeal cells by Ribo Mega-SEC and sucrose density gradient cell fractionation methods

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The _-CASP aRNase J /ASH-Ski2 helicase complex and the RNA exosome are the main RNA-degrading machines involved in RNA surveillance and decay pathways in thermococcales cells. It has been demonstrated that these machines interact with the ribosome [1]. To investigate the relationship between RNA-degrading machines and the ribosome, we developed a new approach called Ribo Mega-SEC, which uses size exclusion chromatography and HPLC to separate polysomes and ribosomal subunits [2]. The Ribo Mega-SEC method offers significant advantages over the more traditional sucrose density gradient technique, including rapidity, reproducibility, and compatibility with subsequent analysis. Furthermore, Ribo Mega-SEC enables direct observation by electron microscopy of polysomes and large protein complexes, as well as functional and structural studies performed on isolated complexes without requiring purification. Isolation of native archaeal ribosomes of Thermococcus barophilus from cells in culture was achieved using two SEC columns in series with different porosity, a 2,000 Å SEC column combined with a 1,000 Å SEC column. Unfortunately, compared to the sucrose density gradient method, the Ribo Mega-SEC did not allow to separate the 50S and 30S ribosomal subunits from the 70S ribosomes in archaeal cells. The ribosomal fractions from Ribo Mega-SEC or sucrose density gradient method were found to contain the majority of aRNase J ribonuclease, ASH-Ski2 helicase, and some RNA exosome subunits. Furthermore, negative stain electron microscopy in these fractions revealed the presence of native ribosomes along with RNA exosome-like structures. The initial findings strongly indicate that RNA-degrading machinery is indeed located near ribosomes during the translation phase, as previously suggested [1]. We will confidently use single RNA exosomes and RNA exosomes associated with ribosomes isolated by Ribo Mega-SEC or by sucrose density gradient cell fractionation methods to successfully solve the Cryo-EM structures of RNA exosome/ribosome complexes in Thermococcales archaea. [1]. Phung, D. K. et al. RNA processing machineries in Archaea: the 5'-3' exoribonuclease aRNase J of the _-CASP family is engaged specifically with the helicase ASH-Ski2 and the 3'-5' exoribonucleolytic RNA exosome machinery. Nucleic Acids Res. 48, 3832–3847 (2020). [2]. Yoshikawa, H. et al. Efficient analysis of mammalian polysomes in cells and tissues using Ribo Mega-SEC. Elife 7, (2018).

The Role of SNORD13 in guiding N4-Acetylcytidine in 18S rRNA: Lessons from Human and Drosophila Models

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Ribosomal RNAs (rRNAs) contain numerous chemically modified nucleotides, the biological and evolutionary roles of which remain only partially understood. These RNA modifications are thought to fine-tune the secondary and tertiary folding of rRNA, thereby shaping ribosome structure and influencing mRNA translation. However, the loss of a single RNA modification rarely results in noticeable phenotypic changes in multicellular organisms. In our recent study, we demonstrated that the highly conserved N4-acetylcytidine (ac4C) in helix 45 of human 18S rRNA is synthesized through the guidance of the box C/D small nucleolar RNA SNORD13 (formerly U13). SNORD13 forms two imperfect base-pairing interactions near the target cytidine, likely aiding the recruitment of the N-acetyltransferase NAT10, which catalyzes the cytidine acetylation via mechanisms that remain poorly understood. Surprisingly, SNORD13-dependent ac4C is largely dispensable for human cell growth, ribosome biogenesis, global translation, and zebrafish development. Using Drosophila melanogaster as a model, we identified an atypical SNORD13-like RNA that directs ac4C modification at helix 45 of 18S rRNA, despite lacking one of the two essential antisense elements. This discovery reveals an unexpected flexibility in the mechanisms guiding RNA-directed cytidine acetylation. Moreover, we observed that SNORD13 loss leads to developmental defects in Drosophila larvae under stressful conditions or when exposed to parasitoid wasps (Leptopilina boulardi). These unpublished findings suggest that the absence of SNORD13 and/or its associated ac4C can significantly impact developmental pathways, particularly in challenging environments. References: [1] Bortolin-Cavaille, M. L.; Quillien, A.; Thalalla Gamage, S.; Thomas, J. M.; Sas-Chen, A.; Sharma, S.; Plisson-Chastang, C.; Vandel, L.; Blader, P.; Lafontaine, D. L. J.; Schwartz, S.; Meier, J. L.; Cavaille, J. Probing small ribosomal subunit RNA helix 45 acetylation across eukaryotic evolution. Nucleic Acids Res 2022, 50 (11), 6284-6299. [2] Thalalla Gamage, S*.; Bortolin-Cavaille*, M. L.; Link, C.; Bryson, K.; Sas-Chen, A.; Schwartz, S.; Cavaille, J.; Meier, J. L. Antisense pairing and SNORD13 structure guide RNA cytidine acetylation. RNA 2022, 28 (12), 1582-1596.

RNase J2 is involved in Bacillus subtilis lifestyle choices

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Post-transcriptional control of gene expression is important in bacteria to rapidly adapt to environmental changes. Two ribonucleases play a key role in mRNA degradation in B. subtilis, the endoribonuclease Y and the 5'-3' exoribonuclease J1, both affecting the level of at least 30% of the Firmicute B. subtilis RNAs (Durand et al., 2012). Firmicutes also encode a paralog of RNase J1, called RNase J2, that is mostly found in a complex with J1. The role of RNase J2 in RNA degradation is unclear, however, as it has a very weak 5'-3' exoribonuclease catalytic activity compared to RNase J1. Previous work has shown that, in B. subtilis, a lack of RNase J2 (ΔrnjB) increases the expression of a few genes belonging to the sigD regulon, which governs cell motility (Mader et al., 2008). To better understand the role of RNase J2 in B. subtilis, we first confirmed that RNase J2 deletion directly affects the stability of two mRNAs belonging to the sigDregulon (cheV and motA mRNA). Intriguingly, in contrast to RNase J2 deletion, a catalytic mutant of the enzyme has no impact on the stability of these two RNAs. Our data suggest that RNase J2 targets a specific subset of RNAs for degradation by the catalytic activity of RNase J1. In addition, our last transcriptomic data suggest a far greater role of RNase J2 than previously suspected, with the half-life of hundreds of RNAs altered in the absence of this enzyme. As observed in other Firmicutes (Chen et al., 2015 – Gao et al., 2017), a deletion of the RNase J2 affects motility, in agreement with its impact on the sigD regulon, but also impacts biofilm structure. Interestingly, the level of cyclic-di-GMP, a signaling molecule involved in bacterial behavior such as biofilm formation and motility (Homma and Kojima, 2022), is affected in a strain deleted for RNase J2. This regulation could be mediated by RNase J2 via the upstream regulation of purine synthesis, since RNase J2 affects purR degradation. All together, these results suggest that RNase J2 modulates RNase J1 specificity and plays an active role B. subtilislifestyle decisions.

Durand et al., 2012, Three essential ribonucleases-RNase Y, J1, and III-control the abundance of a majority of Bacillus subtilis mRNAs. PLoS Genet Mader U, Zig L, Kretschmer J, Homuth G, Putzer H. mRNA processing by RNases J1 and J2 affects Bacillus subtilis gene expression on a global scale. Mol Microbiol. 2008 Oct;70(1):183-96. doi: 10.1111/j.1365-2958.2008.06400.x Chen X, Liu N, Khajotia S, Qi F, Merritt J. RNases J1 and J2 are critical pleiotropic regulators in Streptococcus mutans. Microbiology (Reading). 2015 Apr;161(Pt 4):797-806. doi: 10.1099/mic.0.000039 Gao, Peng & Pinkston, Kenneth & Bourgogne, Agathe & Murray, Barbara & Hoof, Ambro & Harvey, Barrett. (2017). Functional studies of E. faecalis RNase J2 and its role in virulence and fitness. PLOS ONE. 12. e0175212. 10.1371/journal.pone.0175212 Homma M, Kojima S. Roles of the second messenger c-di-GMP in bacteria: Focusing on the topics of flagellar regulation and Vibrio spp. Genes Cells. 2022 Mar;27(3):157-172. doi: 10.1111/gtc.12921

Role of mutations on the RNA helicase DDX6 in a syndrome of intellectual deficiency associated to a P-body defect.

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The DEAD box helicase DDX6 is involved in various aspects of post-transcriptional regulation ranging from mRNA degradation to translational repression or RNA interference. DDX6 also plays a role in the storage of mRNAs into P-bodies (PB) as one of the essential components to their assembly [1,2]. Recent studies suggest that the storage and translational repression of mRNAs into P-bodies play a crucial role in the regulation of neurogenesis and stem cell expansion, a fine balance required for healthy brain development [3,4]. Our collaborator geneticist Amelie Piton identified five patients with intellectual deficiency (ID) that bear de novo heterozygous missense mutations on 2 evolutionary conserved motifs of DDX6 [5]. Functional studies realized by our team showed a causative link between these pathogenic variants and the neurodevelopmental syndrome of the patients associated to RNA misregulation and PB assembly defects [5]. Today, eight more patients have been identified with de novo missense mutations in DDX6. What are the post-transcriptional regulatory functions of DDX6 the most affected by these mutations? What are the underlying mechanisms involved in neurodevelopmental defects? We tackled the first question by investigating the impact of the mutations on DDX6 ability to assemble PB by immunofluorescence experiments performed on fibroblasts derived from patient skin and complementation assays of the new variants in human cell line. Our results show strong defects in PB assembly. We are also investigating the effects of the mutations on the transcriptome of the patients' fibroblasts. On the other hand, in vitro ATPase assays revealed a loss of function for a majority of variants while few of them maintain their enzymatic activity. Currently we aim to investigate the other biochemical activities of DDX6 variants, including interaction with partners. The characterization of molecular and cellular defects of DDX6 variants should eventually shed light on the role of DDX6 and the PB in a context of neurodevelopmental delay. [1] Hubstenberger, A., et al. (2017) Mol. Cell 68, 144-157. [2] Standart, N. and Weil, D. (2018) Trends Genet. 34, 612-626. [3] Bruno Di Stefano, et al. (2019) Cell Stem. 25, 622–638. [4] Kedia S, et al. (2022) Cell Reports. 40, 111070. [5] Balak, C., et al. (2019) Am. J. Human Genet. 105, 3, 509-525

CHARACTERIZING THE RIBOSOME INTERACTOME OF MURINE BONE MARROW-DERIVED MACROPHAGES UPON ACUTE INFLAMMATORY STIMULATION TO UNCOVER NEW REGULATORY PLAYERS.

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Although transcriptional regulation has been at the forefront of studies of innate immunity, the role of post-transcriptional regulation (such as alternative splicing, mRNA decay or translational control) in controlling gene expression in macrophages and other innate immune cells is emerging as an important regulatory layer. Among these, regulation of mRNA translation allows for rapid and reversible modulation of gene expression but its precise role and control mechanisms in the inflammatory response remain poorly understood. This study aims at characterizing the role of ribosomes and mRNA translation in regulating the inflammatory response. In particular, we propose to identify the complete set of of ribosome associated proteins (RAPs) and to determine their role in the context of “specialized ribosomes” with specific regulatory activities using a novel transgenic mouse model expressing Flag-Tagged ribosomes. Bone marrow-derived macrophages (BMDMs) from RPS17-tag and wild-type (WT) mice were differentiated and characterized phenotypically. Flow cytometry was utilized to analyze cell surface markers (CD45, CD11b, F4/80, CD11c). Phagocytosis assays with fluorescein conjugated Saccharomyces cerevisiae were conducted to assess macrophage effector functions. Mass spectrometry was then employed for proteomic analysis of immunoprecipitated ribosomal complexes following LPS or IFN-gamma stimulation. We first validated the suitability of the RPS17-Flag-8His transgenic mice line as a model to study bone marrow derived macrophages. We then identified by mass spectrometry novel RAPs significantly enriched in pro-inflammatory macrophages, indicating a coordinated regulation of ribosome-associated proteins in immune response. This study suggests a potential mechanism of localized translation in response to inflammatory stimuli. Future research should explore the kinetics of ribosomal complex remodeling and the mechanistic interactions of identified RAPs, contributing to a deeper understanding of translational regulation in innate immunity.

Ribosomal RNA Pseudouridylations are Driven by Loose Regulation of snoRNA Abundance

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The discovery of ribosome heterogeneity has introduced the concept of distinct ribosomal subpopulations within the same species. They can be identified through variations in structural elements including ribosomal proteins, ribosomal RNA (rRNA) sequences and post-transcriptional modifications [1]. In humans, those modifications are mainly pseudouridylations and 2'-O-methylations and ensure the assembly and functioning of ribosomes. Pseudouridylations are performed by small nucleolar complexes where non-coding small nucleolar RNA (snoRNAs) from the H/ACA class act as sequence-specific guide for the modification. Therefore, expression levels of snoRNAs are often expected to determine the levels of rRNA modifications. Our findings revealed that the snoRNAome is not fixed and shows variable levels of abundance across tissues [2]. Another article of our team unveiled the association between a systematically overexpressed snoRNA and an increasing level of pseudouridine in different phenotypes of ovarian cancer [3]. However, the relationship between snoRNA abundance and rRNA modification levels has never been systematically examined, leaving mechanisms of rRNA editions unclear. Here we used a set of twelve various tissues including stem cells to examine the link between snoRNAs and rRNA modifications. Sequencing snoRNAs by TGIRTseq and monitoring pseudouridylation levels by HydraPsiSeq in the same samples enabled the mapping of snoRNA and pseudouridylation profiles to compare their levels within and between tissues. Distinct pseudouridylation profiles across tissues are defined by a subset of less stable sites as most sites remain highly modified. These completely modified sites, located near the ribosome's catalytic centers are linked to specific snoRNAs families whose abundance still varies widely suggesting a non strictly snoRNA dependent control on pseudouridylation levels. Strong correlation between snoRNA abundance and modification was seen at a few tissue-specific sites, indicating that snoRNA levels may still influence some modifications. Overall, modification sites, whether complete, partial or variable, and their limited correlation with snoRNA expression point to a complex regulatory system where additional factors such as flexibility might have allowed snoRNA to gain extraribosomal functions without disrupting their original core role in the ribosome. [1] M. Taoka et al., “Landscape of the complete RNA chemical modifications in the human 80S ribosome,” Nucleic Acids Res., vol. 46, no. 18, pp. 9289–9298, Oct. 2018, doi: 10.1093/nar/gky811. [2] e. Fafard-Couture, D. Bergeron, S. Couture, S. Abou-Elela, and M. S. Scott, “Annotation of snoRNA abundance across human tissues reveals complex snoRNA-host gene relationships,” Genome Biol., vol. 22, no. 1, p. 172, Dec. 2021, doi: 10.1186/s13059-021-02391-2. [3] L. Faucher-Giguere et al., “High-grade ovarian cancer associated H/ACA snoRNAs promote cancer cell proliferation and survival,” NAR Cancer, vol. 4, no. 1, p. zcab050, Mar. 2022, doi: 10.1093/narcan/zcab050.

Genome-wide mapping of the targets of Rho-dependent termination of transcription in Mycobacterium tuberculosis

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Bacterial fitness requires timely and faithful gene expression, which relies not only on the accurate initiation and elongation of transcription but also on its proper termination. By defining the 3' termini of nascent RNAs, transcription termination regulates the expression of the transcribed genes while preventing inappropriate transcription read-through the downstream regions. Bacteria use two main pathways to terminate transcription. RNA polymerase alone can stop RNA synthesis at intrinsic terminators whereas additional proteins are necessary to trigger transcription termination at factor-dependent terminators. A major termination factor is the RNA helicase/translocase Rho. In Escherichia coli, Rho-dependent transcription termination has many roles, including surveillance of the coupling between transcription and translation, silencing of foreign DNA or pervasive antisense transcription, and prevention of conflicts between the transcription and replication machineries [1]. While Rho-dependent termination has been studied extensively in E. coli, there are significant variations in Rho features across taxa. This raises the question of whether Rho-dependent termination is conserved across species, in particular in bacteria phylogenetically distant from E. coli. In the major pathogen Mycobacterium tuberculosis, Rho is essential despite unusual sequence and activity features. For instance, a single-point substitution of leucine to methionine at position 495 in the C-terminal domain of M. tuberculosis Rho (MtbRho) as compared to E. coli Rho (EcRho) weakens its ATPase activity [2]. However, this substitution also makes MtbRho resistant to Bicyclomycin, a natural antibiotic that inhibits Rho in other species [3]. The MtbRho protein also contains a large insertion domain in its N-terminus (N-terminal Insertion Domain, aka NID), which governs Rho activity and interaction with RNA by an unknown mechanism [2]. To better characterize Rho-dependent termination in M. tuberculosis, we aimed to identify Rho utilization (Rut) sites at genome scale and determine the role of the NID domain in Rut site selection. To this end, we have implemented a new screening approach, Helicase-SELEX, which is immune to the posttranscriptional RNA processing events that usually obscure the detection of Rho-dependent termination loci by standard transcriptomics [4]. Here, I will explain the general principle and advantages of Helicase-SELEX and discuss our achievements to date. I will also describe our strategy to explore the features and role of the NID domain in Rut site selection using a combination of biochemical and structural approaches. References: [1] L. Bossi, N. Figueroa-Bossi, P. Bouloc, M. Boudvillain, Biochim. Biophys. Acta BBA - Gene Regul. Mech. 1863 (2020) 194546. [2] F. D'Heygere, A. Schwartz, F. Coste, B. Castaing, M. Boudvillain, Nucleic Acids Res. 43 (2015) 6099–6111. [3] E. Saridakis, R. Vishwakarma, J. Lai-Kee-Him, K. Martin, I. Simon, M. Cohen-Gonsaud, F. Coste, P. Bron, E. Margeat, M. Boudvillain, Commun. Biol. 5 (2022) 120. [4] M. Delaleau, E. Eveno, I. Simon, A. Schwartz, M. Boudvillain, Proc. Natl. Acad. Sci. 119 (2022) e2209608119.

Methyl donor deficiency, Ube3a and regulation of gene expression by epigenetic modifications.

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UBE3A is the gene responsible for Angelman syndrome (AS), a disorder characterized by developmental and neurological defects, leading to intellectual deficiency. Its expression is regulated by imprinting and in neurons, expression is solely driven from the maternal allele. A long antisense-transcript for UBE3A is produced from the unmethylated paternal allele, thus blocking expression of UBE3A, whereas methylation of an imprinting center on the maternal allele precludes production of this antisense transcript. Methylation of DNA (one of the most characterized mechanism involved in imprinting) is regulated by the level of S-adenosylmethionine (SAM), the universal methyl donor group used for all transmethylation reactions in the organism. SAM is produced through the one-carbon metabolism, that is dependent on sufficient nutritional supply of both vitamins B9 and B12. Deficiency in both vitamins is frequent in pregnant women and elderly people and is associated with increased risks of neurological defects, some of them being similar to those observed in AS. In addition, patients with rare inborn errors in processing enzymes of the one-carbon metabolism, also present with similar neurologic symptoms. Using a methyl donor deficient (MDD) rat nutritional model, we showed that Ube3a expression is specifically decreased in male pups born to dams fed the MDD diet. By using both the rodent and cellular models, we studied how MDD can affect UBE3A expression and subsequently modulate expression of miRNAs belonging to the Dlk1-Dio3 locus, with potential downstream effects on histone methylation. Taken altogether, our results suggest a link between UBE3A, miRNAs and histone modifications.

Transcription and mRNA processing recovery after global Pol II promoter proximal blocking

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We have recently developed technology to isolate authentic intact nascent transcripts associated with transcribing RNA polymerase II (Pol II) complexes, called POINT technology (Sousa-LuÃ_s at al., 2021). This has allowed us to isolate and study pre-mRNA undergoing mRNA processing, without the use of potentially interfering modified nucleotides. We have now combined our POINT technology with the Padgett technique that exploits the reversibility of the CDK9 inhibitor DRB (see also poster #19). Upon DRB treatment, Pol II is blocked (synchronised) at proximal promoter pause sites. DRB washout then allows transcription to restart from the same sites in all cells, so that pre-mRNA begins to be produced. A time course of Pol II release from proximal promoter pausing (by DRB washout) affords the study of both co-transcriptional splicing and 3'end processing. We observe that whilst transcription recovers quickly after DRB washout, splicing and 3'end cleavage need more time to restart. This also causes a substantial transcriptional readthrough effect. We further demonstrate that DRB does not directly interfere with the splicing reaction. Instead, long DRB-treatment affects Pol II CTD phosphorylation patterns, which in turn prevent spliceosome recruitment to elongating Pol II complexes. Notably, we identify an increase in CTD Y1P after DRB washout, concomitant with splicing deficiency. Subsequent Mass Spec analysis shows that spliceosome components do not interact with Y1P Pol II. This suggests a possible role for Y1P in defining spliceable regions in transcription units. To conclude, our work shows that long DRB treatment, followed by a withdrawal of the drug uncouples transcription and mRNA processing.

Disassembly of the 7SK snRNP reshapes the transcriptional landscape of RNA polymerase II

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Promoter-proximal pausing is a key regulatory step in the transcription of protein-coding genes by RNA polymerase II (RNAPII) [1]. The transition to productive elongation relies on P-TEFb, a kinase-containing complex that promotes synthesis of full-length messenger RNAs through releasing paused RNAPII into gene bodies [2]. P-TEFb activity is primarily controlled by the 7SK small nuclear RNP (snRNP) composed of the 7SK snRNA, LARP7 and MePCE. Together with HEXIM1, the 7SK snRNP sequesters a fraction of nuclear P-TEFb into a catalytically inactive 7SK/HEXIM/P-TEFb snRNP, thus reducing the cellular availability of P-TEFb [3]. In response to various stresses, the 7SK/HEXIM/P-TEFb snRNP is rapidly disassembled, reinforcing the active pool of P-TEFb [4]. However, the influence of increased P-TEFb activity on RNAPII transcription remains to be fully elucidated. Here, we use a degron approach to assess the functional consequences of rapid 7SK snRNP disassembly on RNAPII transcription and gene expression. We show that acute depletion of LARP7 or MePCE, the two 7SK chaperones, leads to the release of P-TEFb from the 7SK/HEXIM/P-TEFb snRNP. Released P-TEFb is rapidly recruited to chromatin, suggesting that the main function of the 7SK RNP is to sequester P-TEFb away from transcription sites. Increased P-TEFb activity has a marked impact on RNAPII pausing genome-wide but more subtle effects on mRNA synthesis, suggesting that not all genes are equally dependent on P-TEFb. We identified a number of genes, some of which encode for stress-response and signal transduction factors, that are significantly up-regulated after 7SK RNP disassembly, most likely because their expression was initially restricted by inefficient pause release. Unexpectedly, high P-TEFb activity can also be detrimental to the expression of other genes that were found to be down-regulated. We will discuss the implications of these results for understanding the transcriptional mechanisms that enable an efficient stress response, as well as for cancer research, given that excessive P-TEFb activity has been reported in a wide variety of cancer cells. [1] L. Core and K. Adelman. Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. Genes & development 33, 960-982, (2019). [2] S. Egloff. CDK9 keeps RNA polymerase II on track. Cell Mol Life Sci. 78:5543-5567, (2021). [3] A. J. C. Quaresma, A. Bugai and M. Barboric. Cracking the control of RNA polymerase II elongation by 7SK snRNP and P-TEFb. Nucleic Acids Res 44, 7527-7539, (2016) [4] C. Studniarek, M. Tellier, P. Martin, S. Murphy, T. Kiss and S. Egloff. The 7SK/P-TEFb snRNP controls ultraviolet radiation-induced transcriptional reprogramming. Cell reports 35, 108965, (2021).

A structural and compositional characterization of the Surf2-5SRNP particle

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The ribosome is one of the most abundant ribonucleoprotein (RNP) responsible for protein synthesis [1]. Its biogenesis is a highly coordinated event inside the nucleus with several hundred proteins and factors involved. Producing ribosomes requires a huge energy investment, which powers maturation factors, production and import of the ribosomal proteins from the cytoplasm to the nucleus, and transcription of the ribosomal RNA (rRNA) by Polymerase I. Interference with ribosome biogenesis promotes signalling pathways that lead to cell cycle arrest, cellular senescence, apoptosis [2], and is associated with cancer, aging, and other age-related degenerative disease [3-6]. Hence, nature has developed different ways to sense the nutrient status, growth factors, extra- and intracellular stress levels, to tightly monitor and regulate this biogenesis mainly by altering the activity of Pol I [7,8]. Starting in the year 2000, reports started to unwind the existence of a fidelity system that quickly halts cell proliferation upon ribosome biogenesis impairment [9], and following studies convincedly showed that tumour suppressor P53 is activated via the binding of several ribosomal components to its negative regulator, MDM2 [10-12]. Among these ribosomal components that can block MDM2 is the trimeric 5S RNP particle composed by RPL5, RPL11, and the 5S RNA [13-14]. In non-stress conditions, the 5S RNA should be integrated into the Large Ribosomal subunit shortly after its genesis, but how does the cell produce the exact same number of copies of 5S and 60S particles, given that both rRNA's are produced by different RNA polymerases (Poll III for the 5S, and Pol I for the 60S)? How does the cell control the accidental production of excess free 5S? Using label free mass spectrometry, we identified SURF2 as a new 5S RNP interacting partner, along with other known names such as MDM2, P53, and SSB. Surf2 is an uncharacterized protein that can be found highly expressed in certain cancers. Over-expression of Surf2 in a U2OS cell-line (osteosarcoma cancer model cell line) renders cells more resistance to drugs that destabilize ribosome biogenesis, possibly because the free 5S particles remain bound to Surf2, preventing a P53 to block the cell-cycle. On the other hand, depleting Surf2 promotes cell cycle arrest, and further renders cells more sensitive to drugs, in a P53 dependent-manner. To better understand the mechanisms underlying the Surf2/5SRNP interaction, we attempted to solve the structure using Cryo Electron microscopy, to unveil the structural determinants and better understand the extra-ribosomal functions of the 5S RNP on cell-cycle arrest, which could encourage the development of drugs especially for cancer, but also ribosomopathies, treatment.

miR-6850 Drives Phenotypic Changes and Signaling in Serous Ovarian Cancer

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Ovarian cancer (OC) is still a major concern in the field of oncology because of its high lethality, due to delayed detection and a therapeutic approach (chemotherapy and cytoreductive surgery) with limited efficacy, owing to the lack of specific druggable targets. These characteristics imply that OC necessitates a more thorough comprehension of the molecular mechanisms guiding both its onset and management. Natural non-coding RNAs called microRNAs (miRNAs) alter post-transcriptional gene expression to affect how the body works. Previous studies have demonstrated a robust association between miRNAs and the initiation and advancement of multiple cancer types, including ovarian, hepatocellular, and breast cancers. Unpublished data from our lab have highlighted the amplification, in one third of serous OC cases, of a genetic region on chromosome 8, encompassing CMYC and RPL8 loci. Importantly, hsa-miR-6850 gene is encoded within intron 1 of RPL8 sequence. In this study, we have examined the expression, function, and potential mode of action of hsa-miR-6850 in cellular models of high grade-serous ovarian cancer (HGSOC). We found that the overexpression of hsa-miR-6850 impacted cellular phenotype at different levels, altering cell cycle, proliferation, and adhesive capacity of SOC cells. Furthermore, miR-6850 facilitated the mesenchymal to epithelial transition (MET) by modifying the expression of several actors of this process. Additional research revealed that overexpression of hsa-miR-6850 affected total protein synthesis and P3IK/Akt/mTOR signaling pathway. Our data indicate that, hsa-miR-6850 may contribute significantly to the development of HGSOC through the P3IK/Akt/mTOR signaling pathway. According to our findings, hsa-miR-6850 might serve, in the future, as a therapeutic target for patients with HGSOC.a

Molecular Basis for the Calcium-Dependent Activation of EndoU Ribonuclease

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Ribonucleases (RNases) are ubiquitous enzymes that process or degrade RNA, essential for cellular functions and immune responses. The EndoU-like superfamily includes endoribonucleases conserved across bacteria, eukaryotes, and certain viruses, with an ancient evolutionary link to the ribonuclease A-like superfamily. Both bacterial EndoU and animal RNase A share a similar fold and function independently of cofactors. In contrast, the eukaryotic EndoU catalytic domain requires divalent metal ions for catalysis, possibly due to an N-terminal extension near the catalytic core. In this study, we investigate the calcium-dependent activation of human EndoU. We determined the crystal structure of EndoU bound to calcium and found that calcium binding remote from the catalytic triad triggers water-mediated intramolecular signaling and structural changes, activating the enzyme through allostery. Calcium-binding involves residues from both the catalytic core and the N-terminal extension, indicating that the N-terminal extension modulates the enzymatic activity in response to calcium. Our findings suggest that similar mechanisms may be present across all eukaryotic EndoUs, highlighting a unique evolutionary adaptation that connects endoribonuclease activity to cellular signaling in eukaryotes.

Identification of neoantigens resulting from aberrant splicing in T-acute lymphoblastic leukemia (T-ALL)

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T-lymphoblastic acute leukemia (T-ALL) is a rare, aggressive type of cancer that develops when lymphoid progenitors proliferate due to a block in their differentiation (thymopoiesis). The progressive accumulation of genetic mutations leads to disturbances in the maturation of T lymphoblasts, affecting their proliferation, survival and differentiation. Although the deregulation of splicing in T-ALL has already been demonstrated, we have undertaken a targeted analysis of the expression of genes involved in T-ALL using long-read sequencing. Our aim is to identify new signatures of this pathology by characterizing new transcripts.

To this end, we have sequenced the transcriptome of T-ALL driver genes in several samples, including different thymocyte stages from healthy individuals, as well as a cohort of 21 patients whose tumor samples were taken at initial diagnosis. The depth of our approach reveals a much broader and more complex transcriptomic landscape than is currently known. Furthermore, we observed an increased diversity of transcripts in patients compared to that detected in healthy thymocytes. In particular, we have found that certain minor transcripts are overexpressed, sometimes by a factor of 20, in some patient samples. We are currently studying these transcripts as a potential source of novel neoantigens, and will confirm their expression on the surface of patient cells by immunopeptidome analysis. The overall aim of this project is to identify new pathological signatures and characterize new neoantigens that can be exploited in immunotherapy.

The challenge to interpret genetic variants mapping to BRCA1 exons 9 and 10 highlights the importance of better understanding the potential rescue role of alternatively spliced transcripts

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BRCA1 is a tumor suppressor gene involved in hereditary breast and ovarian cancer (HBOC). Identifying the causal variant is essential for HBOC diagnosis and for optimal medical care of patients and their families. However, the pathogenicity of certain variants can be questioned. It is the case of BRCA1 c.[594-2A>C;641A>G], a variant responsible for a drastic splicing defect leading to total loss of full-length transcripts (FL) due to out-of-frame skipping of BRCA1 exon 10. Initially interpreted as pathogenic, this variant was re-classified as benign based on a study that revealed a lack of association between the variant and an increased cancer risk. It is since hypothesized that the physiological expression of BRCA1 alternative transcripts lacking exons 9 and 10 (i”9-10) may compensate for the loss of FL transcripts and preserve BRCA1 tumor suppressor function in individuals who carry splicing mutations in these exons. Should this rescue mechanism be confirmed, this would have important consequences on the (re)classification of most variants identified in BRCA1 exons 9 and 10. Here, we provide a comprehensive description of the impact on splicing of 24 variants mapping to BRCA1 exons 9 or 10 by analyzing patients' RNA samples with complementary approaches: (i) fluorescent semi-quantitative RT-PCR coupled to Sanger sequencing, (ii) RT-ddPCR and (iii) allele-specific expression analysis. Moreover, we performed cell-based minigene splicing assays to verify the spliceogenic impact of these variants. Altogether, the results obtained with these approaches were all coherent and allowed to define two sets of variants within our cohort: a set of 15 non-coding variants with no impact on splicing, which can now be classified as probably benign, and a set of 8 variants that induced splicing defects of different severities, including 2 with an impact identical to the one caused by c.[594-2A>C;641A>G]. These variants remain thus of uncertain clinical significance and were prioritized for further studies. We are now: (i) investigating the pathogenicity of the above mentioned spliceogenic variants by collecting and analyzing associated clinical and family data, (ii) extending RNA splicing analyses to other variants within BRCA1 exons 9 and 10, and (iii) developing a CRISPR-based functional assay to better understand the benign character of c.[594-2A>C;641A>G] as well as the potential tumor suppressor role of the alternatively spliced BRCA1 i”9-10 isoform. This study illustrates the importance of better understanding the impact of genetic variants on RNA splicing and of elucidating the biological role of alternatively spliced isoforms of genes implicated in human disorders.

Ribosome profiling of the giant pandoravirus to identify de novo genes during an infection cycle of Acanthamoeba

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In cellular organisms gene birth was thought to mainly occur from duplicated genes or external DNA as raw material, but de novo gene creation from scratch is now seriously considered as a source of genome evolution in a large array of eukaryotic species. In the Pandoraviridae context the following scenario has been drawn to integrate de novo created genes into existing networks. Proto-genes from non-genic regions could gain protein-coding capacity through random mutations, favored by the high GC-content of these genomes. The new open reading frames would stochastically be transcribed, allowing their translation into proteins. Besides their atypical DNA composition, as compared to conserved genes, de novo genes might display specific translation signatures. We will thus compare the translation dynamics of the different Pandoraviridae genes categories using ribo-seq of the pandoravirus genome during the infection cycle. Furthermore, de novo genes are not uniformly expressed during the infectious cycle. They clearly obey to transcriptional regulations, roughly grouped in three classes: early genes mostly involved in infection initiation, intermediate genes in DNA replication and RNA transcription, and late genes strongly enriched in proteins composing the virion particle. Thus, the initial de novo gene expression class might drive their evolution and new function acquisition. We will thus explore the global translational response of P. neocaledonia infected A. castellanii cells in the three main temporal classes compared to uninfected cells. Such an experiment, which has never been done in cells infected by giant viruses, will reveal other translation-related elements. For instance, upstream ORFs, known to be involved in translation regulation, will be screened in both host and viral genes. Likewise, ribo-seq approaches have revealed spurious translation of lncRNAs in cellular organisms, we will thus put particular attention to the potential translation of the Pandoraviridae lncRNAs. This might actually serve has a starting point for de novo gene generation. We will thus question whether this affects the effective translation of the whole system (viral and host genes) or specifically enhance viral genes translation. The first step is to establish the experimental condition to get a good quality of the data set of the ribosomal profiling that will provide information on the ribosome occupancy and dynamics over the infection cycle.

Role of 2'O-ribose methylation (2'Ome) of ribosomal RNAs in early embryonic development

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During mammalian early embryonic development, epiblast stem cells (ECs) acquire pluripotency, the unique capacity to differentiate into all cell types of an organism. This ability is implemented by specific gene expression programs established through epigenetic, transcriptional, and post-transcriptional regulations. Today, many studies have emphasized the central role of translation in integrating upstream transcriptional and post-transcriptional cues to control Pluripotent Stem Cell (PSC) identity. Therefore, a better understanding of translational regulations in PSCs is essential for uncovering molecular processes underlying specific cellular identity and controlling cell fate transitions. Translation is ensured by ribosome, a micromachinery composed of both proteins and RNA (rRNAs). While ribosome composition has long been thought to be invariable, it now appears that ribosomes with different compositions exist, and that these may provide them specialized translational activities. rRNA modifications, such as 2'O-ribose methylation (2'Ome) are an emerging integral part of this heterogeneity. 2'Ome concerns 112 sites in human rRNA, which are specifically guided by small nucleolar RNAs (snoRNAs, or SNORD), often located in key functional sites of ribosomes. We and others contributed to identify 2'Ome heterogeneity in a panel of physiological and pathological contexts. However, whether variations of rRNA 2'Ome occur in PSCs and regulate PSC translation programs by modulating ribosome activity to support underlying cellular properties remain to be explored. In this context, we applied RiboMETH-Seq (a recent high-throughput technique to analyze 2'Ome) in a panel of murine pluripotent and differentiated cells and identified rRNA sites that display differential 2'Ome upon differentiation. Particularly, we found one site to be only 2'O-methylated in PSC, and met first clues suggesting a pluripotent-specific regulation of its snoRNA and the subsequent methylation. It remains now to identify whether such variations are implicated in the pluripotent identity, notably through translational regulation.

Role of mitochondrial translation during T-CD8 responses

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Upon infection, immune responses culminate in the elimination of infected cells, thus limiting pathogen propagation and tissue inflammation. In this context, specialized immune cells harbour the capacity to recognize and kill infected cells through the release of cytotoxic granules, while producing effector cytokines to further strengthen antiviral responses. Among these, professional cytotoxic cells include CD8 T-cells (T-CD8) that represent the frontline of antiviral responses. Their function and memory potential are highly dependent on metabolic changes and rely on a tight coordination between mitochondrial respiration (also known as “oxidative phosphorylation”, OXPHOS) and glycolysis. Naive T-CD8s rely mainly on OXPHOS as a source of energy. Upon encounter with their cognate antigen, T-CD8 effectors undergo a substantial increase of glycolysis which is essential for their effector functions. Finaly, memory T-CD8s are highly dependent on OXPHOS, allowing them to rapidly respond in case of secondary responses. Although those metabolic changes are now well acknowledged, very little is known about the underlying mechanisms that control them. Interestingly, proteins of the OXPHOS complex have a double origin: while most subunits are encoded in the nuclear genome and translated by the classic translational machinery in the cytoplasm, 13 proteins of the OXPHOS complex are exclusively encoded in the mitochondrial genome and translated within mitochondria, in a process called mitochondrial translation. Interestingly, translation in the cytoplasmic and mitochondrial compartments has been shown to be synchronised in order to maintain a correct stoichiometry among OXPHOS subunits. However, the molecular details of this coordinated regulation are poorly understood. Our preliminary results show that inhibition of mitochondrial translation impacts the metabolism, proliferation and activation of T-CD8 in vitro. We are currently generating mice models to investigate the role of mitochondrial translation and the underlying mechanism during T-CD8 responses, in vivo. Altogether, this project should help deciphering the contribution of mitochondrial translation to T-CD8 cytotoxic cells as well as the molecular details of its regulation and coordination with the cytosolic translation machinery.

Live imaging of HIV-1 transcription to understand viral latency

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Combinatorial antiretroviral therapy controls HIV infections. However, therapy interruption leads to viral rebound, necessitating lifelong treatment and posing life threatening risks. Viral rebounds are due to the reactivation of latently infected cells. While the rate at which latent cells are reactivated is a key variable, viral transcription in latent cells is not well characterized. Recent data suggest that latency exit is stochastic, reflecting the random fluctuations of viral transcription observed in live cells. It is hypothesized that some latent cells are not completely silent but sporadically express viral RNA at levels difficult to detect with conventional methods, potentially leading to viral rebounds. Stochastic bursts of viral transcription in latent cells may either activate the Tat feedback loop, fully reactivating the virus, or produce enough viral particles to infect new cells without full reactivation. The MS2/MCP system is a powerful tool to image transcription in live cells with single molecule sensitivity. I first created a two-copy HIV-1 system in HeLa cells, incorporating MS2 stem-loops downstream of the HIV-1 promoter. This system allows differentiation between extrinsic and intrinsic noise sources affecting the HIV-1 promoter. The two-copy cells were imaged for up to 15 hours under basal conditions and after treatment with different categories of latency-reversing agents (LRAs). I observed that transcriptional noise was mostly intrinsic rather than extrinsic, suggesting that viral bursting is mainly due stochastic fluctuation of the viral promoter between different molecular states, some competent for transcription and others not. For more physiological relevance, I incorporated MS2 stem-loops into an HIV-1 dual reporter vector downstream of the HIV-1 promoter. This vector was optimized to reliably isolate latent T cells and image viral transcription in live cells. Several latent clones were imaged for up to 8 hours under basal conditions and upon activation with latency-reversing agents. Under basal conditions, latent clones exhibited bursts of transcriptional activity without exiting latency, with significant cell to cell transcriptional heterogeneity within same clones. These findings provide new insights into the transcriptional kinetics that latent HIV-1 employs to maintain the viral reservoir and to exit latency. Understanding these mechanisms is crucial for developing strategies to eliminate latent reservoirs and achieve a functional cure for HIV.

m6a methylation orchestrates IMP1 regulation of microtubules during human neuronal differentiation

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Neuronal differentiation requires building a complex intracellular architecture, and therefore the coordinated regulation of defined sets of genes. RNA-binding proteins (RBPs) play a key role in this regulation. However, while their action on individual mRNAs has been explored in depth, the mechanisms used to coordinate gene expression programs shaping neuronal morphology are poorly understood. To address this, we studied how the paradigmatic RBP IMP1 (IGF2BP1), an essential developmental factor, selects and regulates its RNA targets during the human neuronal differentiation. We perform a combination of system-wide and molecular analyses, revealing that IMP1 developmentally transitions to and directly regulates the expression of mRNAs encoding essential regulators of the microtubule network, a key component of neuronal morphology. Furthermore, we show that m6A methylation drives the selection of specific IMP1 mRNA targets and their protein expression during the developmental transition from neural precursors to neurons, providing a molecular principle for the onset of target selectivity.

Evolutionary and functional insights into the ASH-Ski2 helicase in Thermococcales

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RNA helicases perform essential housekeeping and regulatory functions in all domains of life by binding and unwinding RNA molecules. Among them, the Ski2-like helicases play primordial role in eukaryotic RNA metabolism pathways. By studying the phylogenetic di- versity of Ski2-like helicases among archaeal genomes and the enzymatic activities of those in Thermococcales, we provided evidence of the function of this protein family in archaeal metabolism of nucleic acids. We showed that ASH-Ski2, previously described to be associ- ated with the aRNase J, 5’-3’ exonuclease, has an unwinding activity with a 3-5 polarity and an annealing activity of RNA duplexes (Batista et al., 2024).

To gain insights into the function of ASH-Ski2, we have analyzed the transcriptome of Ther- mococcus barophilus Δash-ski2 mutant strain and provide evidence of the importance of ASH-Ski2 in cellular metabolism pathways related to translation. Our search for cellular substrates for ASH-Ski2 resulted in the discovery of its importance for G-quadruplexes (G4). G4 structures, formed by guanine rich sequences, are among the most intensively studied lo- cal DNA/RNA structures and regulate key biological processes such as replication and gene expression. A bioinformatics analysis of the genome of the hyperthermophile T. barophilus revealed an important number of potential G4 sequences (PQS). Immunofluorescence exper- iments using the G4-specific antibody, BG4, detected G4s in vivo at the single-cell level with super-resolution microscopy in exponentially growing or stationary T. barophilus cells, and showed the effect of Δash-ski2 mutation on their stability (Aktary et al., 2024). We will address the roles of ASH-Ski2 in the gene expression regulation in Thermococcales.

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

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Box C/D small nucleolar RNAs (SNORDs) are a class of conserved, middle-size noncoding RNAs that form complexes with core proteins to catalyze specific modifications on ribosomal RNA, small nuclear RNA and transfer RNA. Besides this canonical role, some SNORDs are suspected to regulate several levels of gene expression, while around a third of the 1,000 or so human SNORD genes are currently functionally orphan. Here, we present recent findings that SNORDs can act at DNA and RNA levels and that decrypting this information can help us better understand their function. We first focused on multigenic SNORD families associated with pathologies, e.g. rare neurological diseases such as Prader Willi syndrome for the SNORD115 and SNORD116 genes or Labrune syndrome for the SNORD118 genes. First, as already described for other gene categories, we collected evidences of neofunctionalization of several SNORD copies 1,2. Second, we found that non-allelic gene conversion events promote genetic homogeneity between paralog copies, particularly when they are genetically colocalized, and propagate single-nucleotide polymorphisms that can be pathological. Third, we observed variable rates of SNORD gene gains and losses with potential consequences for gene dosage; surprisingly, the variation in copy number exhibits evidences of coordination for the neighboring tandem repeats SNORD115 and SNORD116 in several eutherian species, which could be due to a differential chromatin compaction level of parental genomes caused by genomic imprinting 2. Fourth, the analysis of paralog and ortholog sequences can identify selective constraints at nucleotide level, including in human populations, which represents a powerful tool for functional study. As a leading example, a recent characterization of highly conserved hybridization capacities with RNA targets has opened up a new avenue concerning the molecular function of the SNORD116 family 1, the absence of which is strongly suggested to be critical in Prader Willi syndrome. In conclusion, our intention with this work is to initiate an analysis of the functional landscape of the human SNORD genes. In contrast to a static view, the current data draw a picture in which the function of several members of this class of non-coding RNAs is dynamic, composite and emerges from multiplicity. 1. Phylogenetic and Molecular Analyses Identify SNORD116 Targets Involved in the Prader-Willi Syndrome. Baldini L, Robert A, Charpentier B, Labialle S. Mol Biol Evol. 2022. 39(1):msab348. 2. Coordinated evolution of the SNORD115 and SNORD116 tandem repeats at the imprinted Prader–Willi/Angelman locus. Guibert M, Marty-Capelle H, Robert A, Charpentier B, Labialle S. NAR Mol Med. 2024. 1(1):ugad003.

Unraveling the implication of the long non-coding RNA ANRIL in alternative splicing and its impacts on Cancer

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Long non-coding RNAs (lncRNAs > 200-nts, lacking an obvious open reading frame) have emerged as critical players of gene expression at multiple levels of regulation. As key regulators of cellular homeostasis, lncRNAs are increasingly linked to various diseases, including cancer, underscoring the necessity for more detailed investigations [1]. Our team focuses on dissecting the molecular functions of one such disease-associated lncRNA named ANRIL (Antisense Non-coding RNA in the INK4 Locus), whose overexpression has been linked to the aggressiveness and severity of several cancers [2]. ANRIL has been characterized as a significant factor in shaping the chromatin landscape, directly interacting with the genome to influence chromatin features such as H3K27me3 deposition, thereby affecting the expression of multiple genes [3-7]. However, the molecular mechanisms by which ANRIL modulates cellular homeostasis remain poorly understood. By integrating ANRIL genomic occupancy data with transcriptomic profiling of ANRIL knocked-down cells, the team gathered evidence suggesting that ANRIL directly modulates the alternative splicing (AS) of more than 500 genes in HEK293 cells [7]. Given that AS enhances transcriptomic diversity, which may influence oncogenic transformation, and that ANRIL is associated with cancer and modulation of the chromatin landscape - which is closely linked to AS regulation - we aimed to explore the potential functional intersection between ANRIL, AS, and oncogenesis. To this end, we initiated a strategy to precisely characterize the AS events of the genes directly targeted by ANRIL, its protein partners, and the chromatin-associated mechanisms driving AS modulation. We expect that this approach will provide deeper insights into ANRIL's activities in splicing modulation and its impact on cancer progression.

[1] Qian et al., Frontiers in Medicine 7, 2020, DOI: 10.3389/fmed.2020.612393. [2] Sanchez et al., Cancers 15, no 16, 2023, DOI: 10.3390/cancers15164160. [3] Yap et al., Molecular Cell 38, no 5, 2010, DOI: 10.1016/j.molcel.2010.03.021. [4] Kotake et al., Oncogene 30, no 16, 2011, DOI: 10.1038/onc.2010.568. [5] Holdt et al., PLoS Genetics 9, no 7, 2013, DOI: 10.1371/journal.pgen.1003588. [6] Lo Sardo et al., Cell 175, no 7, 2018, DOI: 10.1016/j.cell.2018.11.014. [7] Alfeghaly et al., Nucleic Acids Research 49, no 9, 2021, DOI: 10.1093/nar/gkab245.

G-rich binding mechanism used by translation initiation factor eIF4B

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The importance of single-stranded RNA in translation, and by extension the removal of RNA structure, is evident by the number of helicases and related enzymes required throughout this process, and is implicated in disease when this function is perturbed. A subset of transcripts with structured 5' UTR require the action of the eIF4A helicase for efficient translation initiation and auxiliary proteins are also required for these processes, including the eukaryotic translation initiation factor eIF4B. Due to the large proportion of intrinsic disorder in eIF4B, few structural details have been determined for eIF4B except for the unbound structure of its single folded RRM domain. Using a suite of biophysical approaches, 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, first by the folded RRM domain and subsequently by the intrinsically disordered regions. Using NMR spectroscopy, single-molecule FRET and other approaches we have characterized a surprisingly strong binding preference for guanosine-rich sequences. We have determined an optimal RNA motif for the eIF4B RRM domain with a notable enrichment in guanosine. The RNA affinity is further enhanced by the contribution of the disordered regions and also upon oligomerization and condensate formation. This overall guanosine-centered property aids in the effective helicase activity of eIF4B partners such as eIF4A and we propose a model of single-strand RNA maintenance that is exquisitely suited for guanosine-based binding.

T-cell exhaustion in septic shock and severe COVID-19 patients correlates with site-specific alterations in ribosomal RNA epitranscriptomic marks

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T cell exhaustion plays a central role in sepsis-induced immunosuppression, and deciphering the precise mechanism of this cellular dysfunction could lead to new therapies. In several pathophysiological contexts, the 2'O-ribose methylation of ribosomal RNA (rRNA 2'Ome) has emerged as a level of epitranscriptomic regulation. Here, we report for the first time the site-specific alterations of rRNA 2'Ome epitranscriptomic marks in T cells after sepsis, associated with impaired functionality. Usinga primary human T cells from septic shock and COVID-19 patients, we identified a subset of sites with high inter-individual variability, the level of whicha correlated with lymphocyte effector functions. This was recapitulated in ana ex vivoa model of stimulated T lymphocytes from health donors. Finally, we identified a 2'Ome signature that discriminated samples from septic patients from those of healthy donors. This opens up an entirely new field of research into the molecular mechanisms contributing to T cell dysfunctions following sepsis.

Exploring the autoregulation of NMD transcripts through the study of their 3'UTRs

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Nonsense-Mediated mRNA Decay (NMD) is characterized as a quality control mechanism which targets mRNA harboring premature termination codons (PTC) for degradation. However, recent studies have demonstrated that NMD can also play a broader regulatory role in gene expression by controlling the stability of natural transcripts, thereby shaping the transcriptome to maintain cellular homeostasis. While the canonical pathway targeting PTC-containing transcripts is well understood, the mechanisms governing the degradation of transcripts via their 3' untranslated regions (3'UTR) remain largely unresolved. The protein UPF1 (Up-frameshift 1), the key factor of NMD, plays a central role in mRNA degradation processes, but the process leading to its recruitment on its targets remains elusive. In this study, we confirmed the crucial role of UPF1 in the recognition, targeting and degradation of transcripts based on features present, within their 3'UTRs. Specifically, we showed that the 3'UTR of NMD factor-encoding transcripts regulate their own stability by a mechanism which is not dependent of the exon junction complex (EJC). We therefore propose that additional elements, such as highly structured folded regions within their 3'UTRs, may be necessary to recruit UPF1 and trigger UPF1-mediated decay (UMD) in these transcripts through a 3'UTR-dependent mechanism. This work therefore sheds light on the non-canonical pathways of NMD and brings new data on the molecular features driving UPF1 recruitment on 3'UTR and activation of transcript degradation.

FUBP1 regulates the splicing of long transcripts in human skeletal muscle cells and is required for normal myogenic differentiation.

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FUSE Binding Protein 1 (FUBP1) is a single-strand DNA and RNA binding protein that regulates numerous stages of RNA metabolism (transcription, mRNA stability, translation, and splicing)[1]. First identified as a transcription activator of the c-myc proto-oncogene, FUBP1 has been deeply studied in a growing number of cancers. Recent loss-of-function studies evidenced its role in two differentiation processes, namely hematopoiesis and neuronal differentiation [2, 3]. In skeletal muscle, we have shown that FUBP1 is involved in regulating the splicing of exon 39 of the DMD gene, responsible for Duchenne Muscular Dystrophy when mutated[4]. Then, we were interested in better defining the role of FUBP1 in regulating gene expression and differentiation in skeletal muscle cells. Here, we analyzed gene expression patterns in 3-day differentiated cells from the C25Cl48 human skeletal muscle cell line, comparing siRNA-mediated FUBP1 Knock-Down (KD) and a control siRNA using HTA 2.0 microarrays. We identified 1848 differentially expressed genes and 4998 differentially spliced events in 3145 genes. Functional Enrichment Analysis for alternatively spliced genes revealed an enrichment of pathways related to muscle tissue development, muscle contraction and actin filament organization. Globally, these genes were longer than the mean expressed genes. These results were consistent with the recent work by Ebersberger & colleagues showing that FUBP1 facilitates 3' splice site (ss) recognition and splicing of long introns[5]. Using iCLIP2, we determined the RNA targets of FUBP1 in differentiated cells. FUBP1 binding sites have been identified in 4863 genes, mostly in the introns of large protein-coding genes. These are mainly located at 5'ss and 3' ss, with a distribution varying according to intron length. Interestingly, up to 40% of the genes alternatively spliced in FUBP1 KD were intronic RNA targets of FUBP1. We generated metaprofiles of FUBP1 binding relative to 5'ss and 3'ss in introns flanking up-, down- or not regulated exons to search for specific binding patterns. Finally, we could observe that FUBP1 expression level decreases in differentiating human primary skeletal muscle cells and that FUBP1 KD prevented correct myogenic differentiation.This work evidences the until now not considered role of FUBP1 in the regulation of gene expression and splicing of large transcripts in human skeletal muscle.

[1] Debaize L & Troadec MB. The master regulator FUBP1: its emerging role in normal cell function and malignant development. Cell MolLife Sci, 2019, 76 (2), 259- 281. [2] Zhou W et al., Far Upstream Element Binding Protein Plays a Crucial Role in Embryonic Development, Hematopoiesis, and Stabilizing Myc Expression Levels. Am J Pathol. 2016, 186(3):701-15. [3] Hwang I et al., Far Upstream Element-Binding Protein 1 Regulates LSD1 Alternative Splicing to Promote Terminal Differentiation of Neural Progenitors. Stem Cell Rep. 2018, 10(4):1208-1221. [4] Miro J et al., FUBP1: a new protagonist in splicing regulation of the DMD gene. Nucleic Acids Res. 2015 ,43(4):2378-89. [5] Ebersberger S et al., FUBP1 is a general splicing factor facilitating 3' splice site recognition and splicing of long introns. Mol Cell. 2023, 83(15):2653-2672.

Identification of a bifunctional RNA involved in the virulence of Dickeya dadantii

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Non-coding RNAs play an important role in the regulation of gene expression in a variety range of organisms. In addition to antisense and trans-acting RNAs, which are well described in bacteria, other regulatory mechanisms have been proposed. Some sRNAs have been found to encode peptides, and long transcripts have been found to repress the expression of the overlapping transcripts produced by neighboring genes. This suggests that RNA plays an important role in regulatory networks. We deciphered the transcriptional landscape of the plant pathogen Dickeya dadantii, which causes soft rot disease in a wide range of plant species, including economically important crops such as potato and rice. During our analysis, we identified that several virulence regulator mRNAs overlapped with mRNAs of neighboring genes. The neighboring genes were predicted to encode proteins involved in either metabolism or the stress response. However, their role in bacterial-host interactions remains unclear. The present study focuses on the pecS mRNA, which encodes a transcriptional regulator described to repress the premature expression of virulence factors leading to maceration of the plant tissue. The transcriptional landscape of D. dadantii revealed that the pecS mRNA overlaps with the mRNA of the convergent gene argG at the common terminator region. We hypothesize that the two transcripts may regulate each other through mRNA-mRNA interaction. Since argG is expressed in the absence of arginine and PecS represses virulence factors, the level of arginine may affect the virulence of D. dadantii. Our results show that the amount of the PecS protein is significantly decreased in the absence of arginine and that this is not due to a regulation of transcription initiation. To assess whether increased production of one transcript can titrate the convergent transcript, we overexpressed one of the transcripts and analyzed the effect on the other transcript by quantifying the protein level. The results suggest that the pecSand argG transcripts are bifunctional RNAs that play the role of both mRNAs and antisense RNAs. Finally, in planta experiments are also performed to evaluate the effect of apoplastic arginine on the infection process of D. dadantii.

Specific selection of tRNAs for suppressing nonsense mutations in human genetic diseases

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Premature Termination Codons (PTCs) are frequently involved in severe forms of genetic diseases, amount for 10-20% of them. To overcome these mutations which cause a non- functional protein, drugs administration has been developed to promote PTC readthrough. Among them, our team have already identified 2-guandidino-quinazolin (TLN468) as readthrough inducers on 40 PTCs involved in Duchenne Muscular Dystrophy (DMD) (Bidou L., et al. PNAS 2022). We have now synthesised 60 derivatives of the TLN468 family. For each of them, we have characterised their actions on different PTCs corresponding to mutations found in the DMD (Duchene myopathy) and CFTR (cystic fibrosis) genes. We were able to obtain derivatives 50 times more active than the original molecule. Interestingly some of these derivatives have specific activity on certain stop codons, while others act on all stop codons. Finally, using quantitative mass spectrometry analysis, we were able to demonstrate that certain molecules cause the selective incorporation of a single tRNA depending on the PTC considered. Although the mechanism of action of these molecules is still unknown, this paves the way for choosing the tRNA incorporated by readthrough in the context of treatments.

The RNA-binding protein CELF1 fine-tunes the expression of key transcription factors in ocular lens development and diseases

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Cataract, or ocular lens clouding, is the leading cause of blindness worldwide. Although generally age-related, cataracts can also be of genetic origin, and several cataract susceptibility genes were identified in human or model organisms. Previously we have shown that mice conditionnally inactivated for the gene encoding the RNA-binding CELF1 have early-onset cataract (Siddam et al., PLoS Genet 2018). It is highly probable that cataract in Celf1 cKO mice results from the defective regulation of normal mRNA targets of CELF1. Here, we carried out iCLIP-seq (Individual-nucleotide resolution UV crosslinking and immunoprecipitation - deep sequencing) to reveal the RNA ligands of CELF1 on a transcriptome-wide scale in mouse lenses. This reproducibly identified 286 RNA bound by CELF1 in their 3' untranslated region. The CELF1 binding sites on these newly identified ligands are enriched in UGU trinucleotides, as previously observed in other ligands of CELF1. The set of CELF1 ligands in mouse lens contains 30 mRNA encoding transcription factors. Among them, 4 were previously identified as cataract-related. Immunofluorescence experiments showed that these factors are overexpressed in CELF1-deprived lenses or cultured lens cells. Accordingly, RNA profiling in control and Celf1 cKO lenses revealed that the genes controlled by these transcription factors are also overexpressed. Finally, luciferase reporter assays indicated that CELF1 directly represses the translation of the mRNAs encoding these transcription factors. Together, these data show that CELF1 reduces the expression of key transcription factors in ocular lens, and releasing this repression certainly contributes to the cataract that is observed in the absence of CELF1.

Alternative translation initiation mechanism of Tau mRNAs during Alzheimer's disease

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Tau proteins are known to be mainly involved in regulation of microtubule dynamics. Besides this function, which is critical for axonal transport and signal transduction. Moreover, tau proteins can turn into aggregates and consequently trigger many neurodegenerative diseases termed tauopathies, of which Alzheimer's disease (AD) is the figurehead. Such pathological aggregation processes are critical for the onset of these diseases. My project is part of a collaboration with the Alzheimer & Tauopathies team led by Luc Buee in Lille. Our collaborators have discovered the first N-terminally truncated variant of the Tau protein, whereas all known variants are C-terminally truncated and produced by proteolysis. This shorter variant of 10 amino acids begins at the methionine of codon #11 (Met11-Tau). Interestingly, this variant is N-terminally acetylated by the NatB enzyme, known to interact with the ribosome and target the first methionine. The hypothesis is that the ribosome is capable of initiating translation at codon #11, thus creating an alternative translation initiation site (ATIS). We were first able to demonstrate that translation of the Met11-Tau variant was indeed due to an ATIS and not to a proteolysis mechanism. We determined that translation of this mRNA was cap-dependent, but that the size of the 5'UTR had no impact on translation efficiency. We also determined that the contexts of the two AUG codons #1 and #11 influenced the translation of both Met1 and Met11-Tau versions. Finally, we showed that Met11-Tau translation was not due to leaky scanning of the first AUG codon. Taken together, these results suggest that a non-canonical mechanism of translation initiation and a secondary mRNA structure may be involved in the production of both versions of the Tau protein. This work might contribute to a better understanding of the translation mechanisms involved in Alzheimer's disease and, more broadly, in Tauopathies.

SplicingLore: a web resource for studying the regulation of cassette exons by human splicing factors

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One challenge faced by scientists from the alternative RNA splicing field is to decode the cooperative or antagonistic effects of splicing factors (SFs) to understand and eventually predict splicing outcomes on a genome-wide scale. SplicingLore is an open-access database and web resource that help to fill this gap in a straightforward manner. The database contains a collection of RNA-sequencing-derived lists of alternative exons regulated by a total of 75 different SFs. All datasets were processed in a standardized manner, ensuring valid comparisons and correlation analyses. The user can easily retrieve a factor-specific set of differentially included exons from the database or provide a list of exons and search which SF(s) control(s) their inclusion. Our simple workflow is fast and easy to run, and it ensures a reliable calculation of correlation scores between the tested datasets. As a proof of concept, we predicted and experimentally validated a novel functional cooperation between the RNA helicases DDX17 and DDX5 and the heterogeneous nuclear ribonucleoprotein C (HNRNPC) protein. SplicingLore is available at https://splicinglore.ens-lyon.fr/. Polveche, H., Valat, J., Fontrodona, N. et al. SplicingLore: a web resource for studying the regulation of cassette exons by human splicing factors. Database (2023) Vol. 2023: article ID baad091; DOI: https://doi.org/10.1093/database/baad091

Deciphering how protein synthesis is shaped by CNOT3, the Achilles heel of the CCR4-NOT complex

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Post-transcriptional silencing mechanism control mRNA stability and translation, enabling dynamic and adaptable protein synthesis. This phenomenon mainly relies on the deadenylase activity of the CCR4-NOT complex which can be mobilized by microRNAs, RNA-binding proteins and mRNA modifications. Recent research indicates that CCR4-NOT can target the translating ribosomes through its CNOT3 subunit. This axis could be particularly crucial as CNOT3 is the target of recurrent somatic mutations in numerous pathologies. This is notably the case for a cluster of mutations that targets the N-terminal domain (NTD) of CNOT3 in various tumors, and associated with a high risk of recurrence in pediatric cancers. To understand the function of CCR4-NOT on the ribosome, we generated a human cell line in which CNOT3 is disconnected from ribosomes. Through a ribosome profiling approach, we mapped a repertoire of differentially translated mRNAs in these cells, and found a precise signature of codons sensitive to the presence of CNOT3 on the ribosome, with a predominant selectivity based on the G/C rate at the 3rd position of the codons (GC3). Given that codon usage is not uniform across the transcriptome, and is distinct among mRNAs involved in proliferation (low GC3) or differentiation (high GC3), our results indicate that the absence of CNOT3 on the ribosome could favor the production of a proteome that potentially facilitates proliferation. To test the impact of mutations targeting CNOT3 in this process, we developed interaction assays to evaluate the presence of CNOT3 on the ribosome. Our results showed that a mutation found in over 8% of acute lymphoblastic leukemias completely abolishes the association of CNOT3 with the ribosome, suggesting a translatomic alteration that could lead to the overproduction of a proteome facilitating proliferation. These preliminary data shed light on how a single mutation in CNOT3 can prevent the connection between translating ribosomes and CCR4-NOT to selectively reshapes the proteome.

Ribosome Biology Alterations in High-Grade Diffuse Gliomas: Epitranscriptomic Insights and Therapeutic Opportunities

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Background: High-grade diffuse gliomas (HGGs) are aggressive and heterogeneous tumors that remain largely incurable. Particularly, glioblastomas (GBs) have a poor prognosis, mostly due to treatment resistance and recurrence caused by the heterogeneity and plasticity of tumors cells. Recent studies have highlighted the crucial role of ribosomes biogenesis (RiBi) in cancer progression. Thus, epitranscriptomic modifications of rRNA, such as 2'O ribose methylation (2'Ome), holds a structural and functional importance for ribosome activity and may contribute to translational regulations in various physiological and pathological contexts, including cancer. We hypothesize that variations of ribosome biogenesis including rRNA 2'Ome in HGGs may offer novel diagnostic and therapeutic tools to manage these diseases. Objective: This study aims at investigating ribosome biogenesis and ribosomal RNA (rRNA) epitranscriptomics in IDH wild-type (IDHwt) and IDH mutant (IDHmut) high-grade gliomas (HGGs), with a specific focus on rRNA 2'O-methylation (2'Ome). The goal was to identify novel biomarkers and therapeutic targets by distinguishing different glioma types and analyzing variations between glioblastoma subtypes (classical, mesenchymal, pro-neural) and cells with different degree of stemness and plasticity. Methods: Firstly, we analyzed rRNA 2'Ome using the RiboMethSeq method across 71 HGG samples (IDHwt and IDHmut) and 9 non-tumorous controls. Additionally, we measured ribosome biogenesis by assessing a RiBi signature based on the expression of ribosome biogenesis factors using medium throughput RT-qPCR (Fluidigm). Focusing on GBM, we examined 2'Ome variations across the three molecular subtypes (Mesenchymal, Classical, Pro-neural) using patient derived cell lines and compared 2'Ome status in glioblastoma cells depending on the stemness status, in each molecular subtypes. Results: IDHwt glioblastomas exhibited significant alterations in rRNA 2'Ome at specific sites, while IDHmut HGGs showed a marked increase in RiBi signature compared to non-neoplastic tissues. In addition, our detailed analysis of GBs revealed distinct rRNA 2'Ome signatures across subtypes,with differences relying on the stemness status of GB cells. Conclusion: This study uncovers distinct alterations of the ribosome biology in HGGs, providing new insights into potential therapeutic vulnerabilities that could be exploited in managing these aggressive tumors. Moreover, the study of 2'Ome and ribosome biogenesis as a potential target to prevent relapse and treatment resistance in GBM needs to be pursued as the preliminary data show great results.

Small RNAs and two-component systems connections in Escherichia coli gene regulation

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Bacteria are subjected to numerous stresses and must constantly regulate their gene expression to quickly adapt to their environment. This control can be mediated by different regulators and occur at all stages of gene expression. For instance, two-component systems (TCS) and small RNAs (sRNA) are two classes of widespread regulators in bacteria playing a key role in the response to the environment. While the TCS regulators (also called response regulators) mostly act at the transcriptional level, most sRNAs are post-transcriptional regulators. Furthermore, in model bacteria such as E. coli, multiple examples of mixed regulatory circuits have been reported, where TCSs control the synthesis of sRNAs and, conversely, sRNAs regulate the synthesis of TCSs. In addition, the control of TCSs by sRNAs was found to have different consequences on the TCS targets, and the molecular details are still poorly understood. Hence, the sRNA-TCS regulations are ideal circuits to study the interplay between transcriptional and post-transcriptional control. To study these circuits in more details, we set up a genetic screen to follow the effect of a plasmid library of 96 inducible regulatory sRNAs on the expression of translational fusions to each of the 31 E. coli response regulators. In addition to recovering all previously reported cases of repression of TCS by sRNAs, this screen also unraveled about 15 new regulations. We have validated several of them, among which a sRNA connecting two TCSs, and a new example of TCS feedback control by a sRNA. Together, these data suggest a prevalent role for sRNAs in modulating TCS signaling.

SNORD104-induced ribosomal RNA 2'Ome contributes to Crizotinib resistance in ALK-translocated lung cancer

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While the ribosome has been considered from its discovery as an invariant actor of gene expression, it recently emerged that ribosomal RNA (rRNA) activity is finely regulated by chemical modifications. These rRNA modifications are mediated by another family of non-coding RNAs, the small nucleolar RNAs (snoRNAs), which guide enzymes at specific rRNA sites. We reported that lung adenocarcinoma (LUAD) cells resistant to tyrosine kinase inhibitors (TKIs) display alteration in both their translational program and their snoRNAs and rRNA 2'O-Ribose methylation (2'Ome) profiles. In particular, a concomitant decrease in SNORD104 and 2'Ome at its associated 28S_Cm1327 rRNA site has been detected in cells resistant to a ALK inhibitor. We showed that the dynamic SNORD104-induced abolition of 2'Ome at 28S_Cm1327 has no effect on translation or cell viability in basal conditions, while it affects translation and promotes resistance to Crizotinib. Interestingly, SNORD104 overexpression enhances Crizotinib sensitivity. Overall, our data demonstrate that a single rRNA 2'Ome site plays a key role in cell adaptation to anti-neoplasic drug. They support a role of snoRNA-altered rRNA 2'Ome, as well as of translation, as novel non-genomic mechanism to TKIs resistance in LUAD.

Structural basis of the Integrator complex assembly and association with transcription factors

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Integrator is a multi-subunit protein complex responsible for premature transcription termination of coding and non-coding RNAs. This is achieved via two enzymatic activities, RNA endonuclease and protein phosphatase, acting on the promoter-proximally paused RNA polymerase II (RNAPII). Yet, it remains unclear how Integrator assembly and recruitment are regulated and what the functions of many of its core subunits are. Here, we report the structures of two human Integrator sub-complexes: INTS10/13/14/15 and INTS5/8/10/15, and an integrative model of the fully assembled Integrator bound to the RNAPII paused elongating complex (PEC). An in silico protein-protein interaction screen of over 1,500 human transcription factors (TFs) identified ZNF655 as a direct interacting partner of INTS13 within the fully assembled Integrator. We propose a model wherein INTS13 acts as a platform for the recruitment of TFs that could modulate the stability of the Integrator's association at specific loci and regulate transcription attenuation of the target genes. Razew M., Fraudeau A., Pfleiderer MM., Galej WP. Molecular Cell 2024 https://doi.org/10.1016/j.molcel.2024.05.009

Yeast One hybrid heterologous screening system to identify functional redundancies within Staphylococcus aureus regulators.

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The prevalence of bacterial infections resulting in treatment failure represents over 4.95 million deaths in 2019 [1]. These failures are constantly on the rise, and a quarter of them are due to infections involving bacteria that are multi-resistant to antibiotics (MRB) [1]. These include the ESKAPE group, which includes Staphylococcus aureus. The success of a bacterial infection depends on its ability to adapt to a new environment by regulating the expression of numerous genes. This regulation can take place via protein factors (transcriptional factors, TFs) or regulatory RNAs (sRNAs). However, despite their role in regulation, the genes of these regulators are rarely essential to the survival of S. aureus. However, it has been shown that TFs and sRNAs are entangled in regulatory networks where several TFs regulate the same sRNA and where several sRNAs can have the same target [2, 3]. These functional redundancies may explain the lack of essentiality of these regulators in S. aureus. Such a regulatory relay system would enable S. aureus to better resist aggression or mutation. The aim of this project is to highlight functional redundancies between protein or ribonucleic regulators. Potentially redundant TFs are those regulating the same sRNA targets, or sRNAs regulated by the same TFs. A screening strategy using the heterologous one hybrid (Y1H) system in S. cerevisiae with 6 sRNA promoter regions and 43 TFs from S. aureus was performed. The results were enriched by analysis of available RNAseq data. We found that 20 TFs out of the 43 studied were able to bind at least one of the sRNA promoters, and for one of the promoters we observed the binding of 13 different TFs. Furthermore, as expected, 5 of the 6 promoter regions share at least one TF, and for two promoter regions there is a 66% binding correspondence between TFs. These results support our hypothesis and have enabled us to select pairs of regulators SarA/CodY, NrdR/VraR, GbaA/HssR and srn_9340/SprC that show similar interaction profiles in Y1H. The next objective will be to determine the role of each regulator in the expression of the sRNAs concerned, and then to investigate the phenotypic impact of these regulator duos. The identification of regulator duos essential for growth, virulence, biofilm formation or the development of antimicrobial resistance could, in the longer term, lead to the identification of antibacterial targets.

[1] The Lancet. 2022. Antimicrobial resistance: time to repurpose the Global Fund. The Lancet 399:335. [2] Menard G, Silard C, Suriray M, Rouillon A, Augagneur Y. 2022. Thirty Years of sRNA-Mediated Regulation in Staphylococcus aureus: From Initial Discoveries to In Vivo Biological Implications. Int J Mol Sci 23:7346. [3] Rachwalski K, Ellis MJ, Tong M, Brown ED. 2022. Synthetic Genetic Interactions Reveal a Dense and Cryptic Regulatory Network of Small Noncoding RNAs in Escherichia coli. mBio 13:e01225-22.

Hijacking a lightcycler for enzymology: improvement of a fluorescent bulk assay monitoring helicase activity.

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Helicases are enzymes involved in all aspects of nucleic acid synthesis, regulation and degradation. As a consequence, several methods were developed to monitor their enzymatic activity. In this report, we described an improvement of bulk fluorescent helicase assays to overcome their specific limitations (cost, health and safety regulations, etc..). Using a lightcycler to monitor the fluorescence in real-time, we managed to precisely control the initiation of the helicase reaction through temperature tuning. Therefore, we were able to demonstrate that this setup could provide a qualitative and a quantitative evaluation of the helicase domain of the UPF1 helicase (UPF1-HD) at lower cost compared to other methods. In addition, this approach allows multiplexing, with the monitoring of several fluorophores during the same analysis, which opens up new technical perspectives.

Study of the biogenesis of the Signal Recognition Particle (SRP)

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Signal Recognition Particle (SRP) is a ribonucleoprotein complex which is composed of 6 proteins and one non-coding RNA, the 7SL SRP. The SRP is necessary for the co-translational targeting to the endoplasmic reticulum of secretory and membrane-bound proteins. Mutations within SRP proteins are linked to diseases such as hematological disorders. Despite the importance of SRP, how the particle is assembled in cells and how this mechanism is regulated is still mostly unknown. Several studies support a model in which all SRP proteins, except SRP54, are imported into the nucleolus for assembly with 7SL RNA [1]. The resulting precursor particle is thought to be then exported to the cytoplasm where it incorporates SRP54. Our previous results indicate that additional step(s) of SRP biogenesis occurs in the Cajal bodies [2]. Moreover, we showed that SRP proteins associate with scores of proteins involved in ribosome biogenesis, most of them being nucleolar. Since SRP and ribosomes work together in protein secretion, we are exploring the possibility that their assembly might be coordinated via common assembly factors. My PhD aims to identify and analyze the different complexes formed successively during SRP assembly. In order to accumulate potentially SRP assembly intermediate complexes, I constructed stable cell lines expressing mutated GFP-SRP72 fusion proteins. The mutations were supposed to inhibit the interaction with the 7SL RNA, SRP68, or are present in patients suffering of bone marrow failure and myelodysplasia. We showed that some GFP-SRP72 mutants have defective associations with other SRP proteins, and are mis-localized in cells. One mutant is particularly interesting because it accumulates in the nucleoli and is absent from the cytoplasm. Analysis of the proteomes of the SRP72 mutants revealed potential new assembly factors of SRP biogenesis, and the characteristic of the pre-complexes formed.
[1] Massenet S. In vivo assembly of eukaryotic signal recognition particle: A still enigmatic process involving the SMN complex. Biochimie. 2019;164:99-104. doi:10.1016/j.biochi.2019.04.007 [2] Issa A, Schlotter F, Flayac J, et al. The nucleolar phase of signal recognition particle assembly. Life Sci Alliance. 2024;7(8):e202402614. doi:10.26508/lsa.202402614

The yeast Pby1 decapping co-factor fine-tunes translation by glutaminylating the essential elongation factor eEF1A

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Interactomic analyses have identified Pby1 as a partner of the yeast decapping complex. Consistently, this factor was shown to be a major component of P_bodies. The Pby1 protein contains a SurE domain followed by an ATP_grasp domain. Structural and functional analyses have revealed that the latter binds directly the Dcp2 decapping enzyme, and is thus mandatory for Pby1 recruitment in P_bodies. Surprisingly, however, if the Pby1 ATP_grasp domain could rescue strains genetically impaired in decapping, the potential underlying catalytic activity was not involved in this process. Using phylogenetic data and a candidate_approach, we looked for Pby1 possible role(s) and substrate(s). Using in vivo assays and biochemical reactions in vitro, we demonstrate that the Pby1 ATP_grasp domain catalyzes the highly specific addition of a glutamine on the glutamate E45 side chain of yeast translation elongation factor eEF1A. While this post_translational modification is not essential per se, we show using sensitive assays that removal of Pby1, or of its catalytic activity, affect translation and alter growth of yeast in conditions in which translation is suboptimal. Our results demonstrate that Pby1 harbors a novel enzymatic activity and that it acts at the interface of mRNA decay and translation to fine_tune the latter process.

DHHS1/HAN, an Archaea specific exonuclease, at the interface of DNA and RNA metabolisms

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Archaea are unicellular, prokaryotic and ubiquitous microorganisms that form the third domain of life. Extremophiles Archaea are able to live in environments that question the limits of life. In particular, Thermococcales are hyperthermophiles that develop at temperatures up to 120°C. Maintaining the stability and integrity of their RNAs, at denaturing temperature, is key for these cells, to modulate their gene expression. Our team aims to characterize the RNA degrading machines, of Pyroccocus abyssi and Thermococcus barophilus, that process, degrade and mature the cellular RNAs. The molecular interaction networks of the 5'-3' exoribonuclease aRNase J, of the RNA helicase ASH-Ski2, and of Rrp41 and Csl4, two subunits of the RNA Exosome, with 3'-5' exoribonucleolytic activity, have been established ([1]; unpublished results). At the center of these networks, a new nuclease, DHHS1/HAN, of unknown function, have been discovered. The protein belongs to the superfamily of the DHH phosphodiesterases that are involved in fundamental processes, such as DNA replication and repair, RNA degradation and nucleotide metabolism. Our phylogenomic studies showed that DHHS1/HAN belong to a DHH family, specific to Euryarchaea. This family is defined by its N-terminal domain, that contains both nucleic acid and protein binding motifs. We also determined that DHHS1 has a 3'-5' exonuclease activity in vitro on ssRNA and ssDNA. Interestingly, while the exonuclease have the ability to degrade DNA and RNA, it can also degrade both strands of DNA:DNA with 3' overhangs, but it cannot fully degrade an RNA strand embedded in a RNA:RNA duplex. Affinity pulldown experiments, using DHHS1/HAN as bait, confirmed the presence of the RNA players aRNase J, ASH-Ski2, Rrp41 and Csl4 in its molecular interaction network. We also showed that it is its N-terminal domain that is responsible for forming this network. Moreover, copurification assays indicate that DHHS1/HAN might interact with ASH-Ski2 and aRNase J. Finally, as in Pyrococcus furiosus[2-3], in P. abyssi, we established a link between DHHS1/HAN and Hef, a DNA helicase/endonuclease that is involved in stalled replication fork restart[4]. Hef is homologous to eukaryotic FANCM that can unwind RNA:DNA hybrids[5]. Interestingly, we also uncovered a relationship between Hef and aRNase J. Altogether, these data suggest that DHHS1/HAN plays a role at the interface of DNA and RNA metabolisms. We now want to investigate if DHHS1/HAN is involved in DNA processes. To do so, the substrates of DHHS1/HAN will be determined by CRAC (cross-linking and cDNA analysis) and ChIP (Chromatin immunoprecipitation) followed by high-throughput sequencing. We are also investigating if DHHS1/HAN is involved in the processing of RNA:DNA hybrids by performing DRIP (DNA-RNA immunoprecipitation) experiments in wild type and ΔdhhS1 cells.

Does 53BP1 bind to RNA-DNA primers to prevent the activation of innate immune response ?

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Genomic instability has many incomes, either endogenous or exogenous sources. In addition to pathogenic DNA, self-DNA, which is accumulated in cytosol, can activate innate immune responses, leading to the production of type 1 interferon (IFN) stimulated genes (ISG). This activation would be mediated by the cGAS-STING pathway. The team has recently found that 53BP1 protein binds to RNA-DNA primers in unperturbated DNA replication (Leriche et al., 2023).
We ask whether the binding activity of 53BP1 can play a protective role against uncontrolled release of RNA-DNA chimeras. For example, we have shown that depletion of 53BP1 activates expression of many markers of inflammatory type 1 interferon.

Study of the structural rearrangements of the 5'-untranslated region of the HIV-1 genomic RNA during virion maturation.

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The Human immunodeficiency virus type 1 (HIV-1) is a Retrovirus packaging a dimeric form of its RNA genome (gRNA). The highly structured and conserved 5'-untranslated region (5'-UTR) plays a crucial role in many stages of the viral cycle, including gRNA selection, packaging and maturation. Maturation of the HIV-1 viral particles shortly after budding is required for infectivity. During this process, the Pr55Gag precursor undergoes a cascade of proteolytic cleavages, and whilst the structural rearrangements of the viral proteins are well understood, the concomitant maturation of the gRNA structure is unexplored, despite evidence that it is required for infectivity. To better understand the process of structural rearrangement of the gRNA dimer during maturation, we studied in vitro the interactions between the Pr55Gag protein and its maturation products (NCp15, NCp9 and NCp7) and the 5'-end of the gRNA. The structural rearrangements induced by the binding of each of these proteins were studied by a solution-based chemical mapping approach called hSHAPE (high throughput Selective 2'-Hydroxyl Acylation analyzed by Primer Extension). The effects of the binding of each of these proteins on the RNA structure could be highlighted by comparing the data obtained for the gRNA dimer alone or in the presence of one of the proteins. Our results show that Pr55Gag and its maturation products mostly bind at different RNA sites and with various contributions of their two zinc knuckle domains. Importantly, these proteins have different transient and permanent effects on the RNA structure, the late NCp9 and NCp7 maturation products inducing dramatic structural rearrangements. This study enabled us to highlight the contribution of each protein and to propose a model for the structural rearrangement of the 5'-UTR of HIV-1 gRNA during viral particle maturation.

Combination of a novel miRNA signature with CEA and CA19-9, an approach to improve the efficacy of colorectal cancer detection and prognostication

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Background and aims:
Colorectal cancer (CRC) is the third most prevalent cancer type and the second leading cause of cancer-related deaths worldwide (1). The alarming increase in death numbers and incidence of early-onset colorectal cancer (CRC) emphasizes the improvement of existing biomarkers or developing novel indicators of CRC behavior (2, 3, 4). This study aims to develop a new panel of miRNAs as a diagnostic and prognostic signature for CRC patients compared with the standard tumor markers, CEA and CA19-9.
Material and methods: Using qRT-PCR, the expression levels of a panel of miRNAs including miRNA-103a-3p, miRNA129-5p and miRNA27a-3p were determined in serum and tissue samples from CRC patients (n=40) compared to cases with intestinal polyps (n=10) and healthy controls (n=50).
Results: Serum and tissue expression of miRNAs were higher in patients with advanced CRC, lower tumor differentiation, and larger tumor size, as compared with the other partici- pants ($p < 0.05$). Moreover, this miRNA signature served as a promising biomarker for early detection (AUC: 0.97, $P < 0.0001$), and staging of CRC patients (AUC: 0.96, $P < 0.0001$); while in combination with CEA and CA19-9 could reliably predict metastasis (AUC: 0.95, $p=0.0001$). Additionally, expression levels of tissue miR-129-5p and 27a-3p could distinguish between polyp tissue and the corresponding normal one, whereas serum miR-27a-3p levels could differentiate CRC samples from intestinal polyps.
Conclusion: This miRNA signature could improve the accuracy, sensitivity and specificity of CRC detection and prognostication and thereby better patient stratification.

Disordered regions of translation initiation factor eIF4B orchestrate a delicate balance across monomer-oligomer-condensate landscape

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Eukaryotic translation initiation factor eIF4B is essential for efficient cap-dependent translation, particularly for mRNAs with extended and structured 5' untranslated regions. It is tightly regulated to ensure proper physiological functions and responses, but is frequently dysregulated in various pathologies. Despite its significant functional importance, eIF4B is rarely observed in cryo-EM structures of translation complexes due to its high intrinsic disorder. As a result, the molecular details of eIF4B and especially its long intrinsically disordered region (IDR) remain largely unknown. By integrating experiments and simulations we demonstrate that eIF4B IDR orchestrates and fine-tunes an intricate transition from monomers to a condensed phase, in which variable-size dynamic oligomers form before mesoscopic phase separation. Interestingly, under certain conditions the oligomer population displays a pronounced bimodal size distribution, consisting of minimal dimers and relatively small oligomers alongside larger multimolecular oligomeric clusters. These larger oligomeric assemblies act as nucleation hot-spots and are essential for observation of phase separation-driven condensation. The observed complex self-association landscape displays strong sensitivity to ionic strength and molecular crowding, which suggests potential regulation of eIF4B self-association, such as driven by protein modifications or changes to the cellular environment, as a fine-tuned mechanism for its cellular function.

Optimizing mRNA Delivery: Maltodextrin-Modified lipoplexes for Nasal Administration

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mRNA vaccines offer a novel platform for inducing antigen-specific immunity by enabling recipient cells to express target antigens, thus bypassing traditional antigen production methods. This study explores the use of maltodextrin (MDX) as an adjuvant to enhance the delivery of mRNA-loaded lipoplexes (LXs) for mucosal administration. Mucosal vaccination, particularly via the nasal route, is an attractive strategy for eliciting robust immune responses at pathogen entry points. Further investigations into the colocalization of MDX and lipoplexes using confocal microscopy and cryogenic electron microscopy confirmed the successful incorporation of MDX, which significantly improved transfection efficiency and gene expression in airway epithelial cells. We demonstrate that MDX-coated LXs significantly improve mRNA delivery and expression in epithelial airway cells, with a notable four-fold increase in protein expression sustained for up to 48 hours post-administration. The incorporation of MDX enhances the stability of LXs and their interaction with the mucosal barrier, leading to improved uptake in the presence of mucin. While the exact mechanism remains to be fully elucidated, it is hypothesized that MDX reduces LX hydrophobicity, facilitating mucus penetration and cellular uptake. These findings validate the potential of MDX-coated LXs for nasal gene therapy applications and pave the way for further research into optimizing mucosal vaccine delivery systems.

Transcriptional adaptation during vertebrate development at the single-cell level

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The development of a multicellular organism requires the precise control of gene expression in space and time so that cells adopt their correct identity. However, genetic mutations can alter this complex process. Recently, transcriptional adaptation (TA) has been uncovered as one of the mechanisms underlying genetic compensation in zebrafish, mouse cells in culture, and Caenorhabditis elegans. TA refers to the phenomenon by which mutated genes (often with mRNA-destabilizing mutations) trigger the transcriptional modulation of related genes, called adapting genes. However, little is known about the spatial and temporal characteristics of adapting gene regulation and particularly during zygotic genome activation. My project aims to decipher when and where TA occurs during early zebrafish development. Using genome engineering followed by live imaging, high-resolution microscopy and quantitative analysis, we investigate the temporal feature of the TA response during the zygotic genome activation. We generated zebrafish lines suited for live imaging of transcription to then study the specific mode of transcription during the modulation of the adapting genes (i.e., linear/discontinuous). Furthermore, we will investigate the subcellular localization of mutant mRNA degradation as well as potential factors involved in the TA machinery. Until now, TA has been mostly investigated on pooled populations of cells. Therefore, we lack the understanding of this phenomenon at the single cell level. This project aims to fill this gap and obtain a better understanding of the spatio-temporal characteristics of genetic compensation which aid in the robustness of vertebrate development.

Beyond tRNA splicing and No Go mRNA Decay, the Importance of the RNA Kinase Trl1

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The No-Go Decay (NGD) mRNA surveillance pathway degrades mRNAs containing stalled ribosomes during the elongation step of translation. NGD occurs when ribosomes are blocked by the presence of stable intra- or intermolecular RNA structures, enzymatic cleavage, chemically damaged sequences, or rare codons. Other mRNA surveillance pathways can also ultimately lead to NGD. For instance, transcripts synthesized without a stop codon due to premature polyadenylation have stalled ribosomes that are initially detected by the Non-Stop Decay (NSD) pathway. NSD targets mRNAs are cleaved and become targets of NGD when ribosomes reach the new 3'-end and stall. NGD thus plays a key role in resolving translational issues potentially detrimental to cellular homeostasis.

These problematic mRNAs can be degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. When ribosomal collisions are too robust, Cue2-mediated endonucleolytic cleavage activity can serve as a fail-safe system. Cue2 displays an SMR (small MutS-related) hydrolase Cter-domain and two conserved CUE ubiquitin-binding domains, allowing its recruitment to collided ribosomes after the ubiquitination of specific ribosomal proteins by the ubiquitin ligase Hel2. Cue2 cleavage proximal to the stalling site results in the production of 5_ NGD and 3_ NGD_RNA intermediates. 5_ NGD RNA intermediates are degraded by the 3'-5' exonucleolytic pathway, which involves the Ski complex (Ski2, Ski3 & Ski8), Ski7, and the cytoplasmic exosome, while the 3_ NGD_RNA intermediates are degraded by the 5'-3' Xrn1 exoribonuclease.

Several years ago, we focused on 3' NGD-RNA intermediates and demonstrated that these RNAs were 5'-hydroxylated. Surprisingly, these intermediates, now known to be produced by Cue2, are degraded by the 5'-3' Xrn1 exoribonuclease, which exclusively requires a 5'-monophosphorylated extremity for degradation. Our investigation revealed that the RNA kinase activity of Trl1 is responsible for the phosphorylation of these 5'-hydroxylated RNAs generated by NGD.

Trl1 is a well-characterized factor with RNA kinase, cyclic phosphodiesterase activity, and ligase activities essential for tRNA processing. Indeed, splicing of tRNAs is known to generate 5'OH-intronic RNAs, which require Trl1 kinase activity to permit their degradation by Xrn1. Here we show that its RNA kinase activity is not limited to tRNA splicing and NGD-targeted mRNAs but also degrades the majority of 5'-hydroxylated RNA species present in the cell. The importance and cellular advantage of such RNA kinase activity are discussed.

The impact of RNA modifications on the translation fidelity

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Like DNA and proteins, RNA is subject to chemical modifications that can alter RNA metabolism like folding, stability, splicing, nuclear export, location and translation. Over a hundred chemical modifications have been identified in RNA, mostly in tRNA and rRNA but also in mRNA. However, the role of these modifications on translation is still unclear. In particular, the impact of mRNA and tRNA modifications on recoding events such as readthrough, during which tRNAs are incorporated at the stop codon, allowing translation to continue until the next stop codon, has not been investigated yet. To test the impact of mRNA modifications on translation accuracy, we are synthesizing mRNAs based on Glutathione S-Transferase (GST) sequence, carrying a stop codon in a readthrough context at the beginning of the coding sequence. In this way, GST is only produced after a readthrough event. These mRNAs are constructed in several parts. The region including the modified base is chemically synthesized; the rest of the sequence is in vitro transcribed. The two fragments are then ligated together to form the complete GST mRNA sequence. The readthrough efficiency is estimated with the quantity of the GST proteins produced in presence or absence of modification. The GST proteins are then analyzed by mass spectrometry to identify any change in amino acids due to the modified nucleotide. tRNAs are the most highly modified RNAs in cells. However, it is difficult to assess the impact of modifications on the ability of tRNAs to decode the corresponding codon, because in most cases, cognate tRNAs are efficiently incorporated regardless of their modification. To circumvent this problem, we chose to study the incorporation of tRNAs at a stop codon, during a readthrough event in human cells in culture. We could highlight the complex interrelationship between two modifications present in the anticodon loop of the human tyrosine tRNA. While Psi35 appears to be essential for the decoding of both tyrosine codons and UAG/UAA stop codons by the tyrosine tRNA, we were able to show that the GalQ modification at position 34, which is specific to human cells, reduces the ability of tyrosine tRNA to be incorporated as a near-cognate. As the precursor of GalQ34 is mainly provided by the microbiota, this suggests an important role of the human microbiota in the decoding efficiency and translation fidelity.

Structural characterization of an asparagine isomerase involved in the late assembly steps of the small ribosomal subunit

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Ribosomes are essential protein synthesis machines shared by all living organisms and play a crucial role in translating the genetic code. The RNAs and proteins that make up the ribosome assemble in a coordinated and highly regulated manner through a process called ribosome biogenesis. This process involves assembly factors necessary for the folding and modification of ribosomal proteins and RNAs. While our understanding of ribosome biogenesis is now highly detailed, key steps remain to be elucidated. One such step involves the small subunit platform. In this work, we present the structural characterization of a late assembly step of the small ribosomal subunit from E. coli, corresponding to the post-translational modification and positioning of the universally conserved uS11 protein. Using structural biology techniques, I investigated the mechanism of isoaspartylation of this ribosomal protein. After identifying YbeY as the enzyme potentially responsible for the isoaspartylation of uS11, I solved the structure of the complex between YbeY and uS11 using X-ray crystallography. By extending this study to catalytic mutants of the enzyme, I was able to characterize the catalytic mechanism underlying this modification. To further assess the role of YbeY in the positioning of uS11, we solved the structures of pre-ribosomes purified from cells lacking YbeY expression through cryo-electron microscopy. This allowed us to observe the biogenesis defects associated with the absence of this assembly factor and its effect on the integration of uS11.

The REMOTE Project: Unravelling the role of snoRNAs within breast cancer cells and in the formation of a pre-metastatic niche in bone.

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Metastases are the leading cause of breast cancer-related mortality in women worldwide, with bone being the most common site for metastasis (~80%) [1]. Long before being clinically detectable, breast cancer cells from the primary tumour secrete factors into the circulation that remotely control the formation of a fertile soil, the pre-metastatic niche, in bone (or other organs) for the future seeding of disseminated breast cancer cells at these distant sites [1]. Circulating non-coding RNAs, such as microRNAs (miRNAs), expressed by breast cancer cells contribute to the formation of pre-metastatic niches in bone [2]. Small nucleolar RNAs (snoRNAs) are an understudied class of non-coding RNA for their potential role in contributing to breast cancer bone metastasis formation within cancer cells as well as distant regulators [3]. The aim of the REMOTE project is to unravel the functions of snoRNAs in early-stages of breast cancer bone metastasis. We have analysed by nanofluidic-automated real-time qPCR the expression of a panel of snoRNAs in the serum of early-stage breast cancer patients at baseline (time of surgery) whom clinical follow-up information, including subsequent development and the site of metastasis, has been recorded for a minimum of 10 years (Azure cohort) [4]. We have found a downregulation in the expression of several circulating snoRNAs in breast cancer patients with (bone) metastasis compared to those who did not have any distant relapse. Among them, 2 snoRNAs of interest have been selected for future functional studies. Particularly, we aim to further investigate if the modulation of the expression levels of these 2 snoRNAs might be linked to a bone-tropic phenotype of breast cancer cells (by conducting gain- and loss-of-function molecular assays), and if their circulating levels could have an effect on the bone micro-environment (by performing osteoclastogenesis and osteogenic assays after modulation of snoRNA levels). Overall, REMOTE project will identify and characterise snoRNAs that control early-stages of breast cancer bone metastasis formation, and will potentially assist in developing snoRNA-based biomarkers to identify breast cancer patients at a high risk of relapse in bone. Our findings might be extended to other cancers (e.g., prostate, lung) with a high propensity to metastasise to bone. [1] Clezardin, P. et al. “Bone metastasis: mechanisms, therapies, and biomarkers.” Physiological reviews vol. 101,3 (2021): 797-855. doi:10.1152/physrev.00012.2019 [2] Puppo, M., Valluru, M.K. & Clezardin, P. MicroRNAs and Their Roles in Breast Cancer Bone Metastasis. Curr Osteoporos Rep 19, 256–263 (2021). https://doi.org/10.1007/s11914-021-00677-9 [3] Puppo M, Jaafar M, Diaz J-J, Marcel V, Clezardin P. MiRNAs and snoRNAs in Bone Metastasis: Functional Roles and Clinical Potential. Cancers. 2023; 15(1):242. https://doi.org/10.3390/cancers15010242 [4] Coleman RE, Cameron D, Dodwell D, et al. Adjuvant zoledronic acid in patients with early breast cancer: final efficacy analysis of the AZURE (BIG 01/04) randomized open-label phase 3 trial. Lancet Oncol 2014; 15: 997–1006

Circularly permuted 23S rRNA in Thermococcus barophilus

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Circular RNAs are found in all kingdoms of life. This heterogeneous class of RNA molecules has in common their lack of 5' and 3' ends, and their capacity to resist degradation by exoribonucleases that require free RNA termini. In Archaea, an abundant class of circular RNAs correspond to 16S and 23S ribosomal RNA intermediates (circ-pre-rRNAs). They were described in a handful of archaeal organisms by molecular biology techniques and RNA-seq methodologies. A conserved bulge-helix-bulge (BHB) motif within the 16S and 23S rRNAs processing stems and adjacent to the circularization site in Euryarchaeota and TACK superphylum suggests that pre-rRNAs circularization is widely conserved across Archaea. Using genome-wide transcriptomic data obtained on total RNAs of wild-type Thermococcus barophilus cells, we searched for circular junctions by mapping the chimeric reads that align into two segments on the genome. We recovered the canonical circularization junctions of the 16S and 23S circ-pre-rRNAs at the predicted BHB motifs. We also identified three alternative 23S circular junctions that potentially introduce variability at the 3' end of the mature rRNA. Intriguingly, the reads covering the 23S canonical junction are more abundant than the ones corresponding to the 16S junction. It was recently described, in Pyrococcus furiosus, that circularly permuted 23S rRNA is generated during the reopening of the 23S circ-pre-rRNAs. Therefore, our recovered chimeric reads could align to two forms of 23S, the circular intermediate and the permuted linear mature form. We investigated the different forms of 16S and 23S by performing primer extension and RACE experiments. We showed that while the 16S rRNA has standard 5' and 3' extremities, the main form of 23S rRNA is circularly permuted, with helices H99-101 now at its 5' end. This permutation most probably emerged from deleting helix H98 from the 23S circ-pre-rRNA. H98,a only found in a few archaeal genomes, is part of an expansion segment (ES) that expands in Asgard and evolves to be eukaryotic ES39s. Interestingly, the permuted 23S rRNA is incorporated in ribosome subunits and 70S monosomes. It contains an additional sequence corresponding to the BHB junction that fits the cryoEM density map of Thermococcus kodakarensis 50S subunit. It remains to determine the significance of such an event in generating functional 50S particles.

Impact of the autophagic protein GABARAPL1 on arsenite-induced stress granule formation

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The tumor microenvironment is a harsh place that gives a cell a significant number of chances to die. In response to environmental stress (e.g. hyperosmolarity or oxidative conditions), specific kinases like HRI or PERK are activated leading to global translation inhibition by eIF2_ phosphorylation. In this context, untranslated mRNAs condensate into cytoplasmic foci called stress granules (SGs) driven by SG-nucleating proteins like G3BP1 and TIA-1 (Buchan 2014). SGs sequester untranslated mRNAs, translation-related proteins and pro-apoptotic factors. However, certain mRNAs, such as ATF4, are actively translated and contribute to the stress-response, notably by inducing autophagy (B'chir et al. 2013). Autophagy is a cellular stress response process that degrades organelles and proteins through the fusion of autophagosomes with lysosomes. A more specific type of autophagy, known as selective autophagy, targets substrates called “cargo”. This pathway is regulated by receptors from the ATG8 family (Zaffagnini et Martens 2016) which includes GABARAPL1 that was identified by our lab (Vernier-Magnin et al. 2001). Autophagy plays a dual role in SGs dynamics: inhibiting key autophagy proteins, either through genetic knockouts or chemical inhibitors like NH4Cl, has been shown to impair arsenite-induced SGs formation (Seguin et al. 2014), while autophagy is also involved in SGs degradation (Buchan et al. 2013). Our study aims to elucidate the role of GABARAPL1 in the dynamics of SGs. In a model of adenocarcinoma cancer cells, our results indicate that GABARAPL1 depletion impairs SGs formation, in a stress dependent manner. Interestingly, arsenite-treated cells lacking GABARAPL1 are unable to generate SGs, which can be explain by a loss of eIF2_ phosphorylation. We also show that KO GABARAPL1 cells exhibit functional translational machinery, and an increased survival rate. The loss of GABARAPL1 would indicate a potential protective role in cellular stress adaptation and survival, which could be relevant to understanding how cancer cells resist stress. Future studies will examine the precise mechanisms by which GABARAPL1 influences SG dynamics and whether these effects are conserved across different stressors and cancer types. To go further, some anti-cancer treatments (e.g. vincristine, 5-FU, paclitaxel) are known to induce SGs in cancer cells (e.g. breast, lung, colon) and help them to survive. We are currently studying the impact of chemotherapies and radiation therapies on our GABARAPL1 KO inhibition cell models. References B'chir, Wafa, Anne-Catherine Maurin, Valerie Carraro, Julien Averous, Celine Jousse, Yuki Muranishi, Laurent Parry, Georges Stepien, Pierre Fafournoux, et Alain Bruhat. 2013. « The eIF2_/ATF4 pathway is essential for stress-induced autophagy gene expression ». Nucleic Acids Research 41 (16): 7683_99. https://doi.org/10.1093/nar/gkt563. Buchan, J Ross. 2014. « mRNP granules ». RNA Biology 11 (8): 1019_30. https://doi.org/10.4161/15476286.2014.972208. Buchan, J. Ross, Regina-Maria Kolaitis, J. Paul Taylor, et Roy Parker. 2013. « Eukaryotic Stress Granules Are Cleared by Autophagy and Cdc48/VCP Function ». Cell 153 (7): 1461_74. https://doi.org/10.1016/j.cell.2013.05.037. Seguin, S J, F F Morelli, J Vinet, D Amore, S De Biasi, A Poletti, D C Rubinsztein, et S Carra. 2014. « Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly ». Cell Death and Differentiation 21 (12): 1838_51. https://doi.org/10.1038/cdd.2014.103. Vernier-Magnin, Sandrine, Stephanie Muller, Myriam Sallot, Jean Radom, Jean-Francois Musard, Pascale Adami, Philippe Dulieu, Jean-Paul Remy-Martin, Michele Jouvenot, et Annick Fraichard. 2001. « A Novel Early Estrogen-Regulated Gene gec1 Encodes a Protein Related to GABARAP ». Biochemical and Biophysical Research Communications 284 (1): 118_25. https://doi.org/10.1006/bbrc.2001.4908. Zaffagnini, Gabriele, et Sascha Martens. 2016. « Mechanisms of Selective Autophagy ». Journal of Molecular Biology 428 (9Part A): 1714_24. https://doi.org/10.1016/j.jmb.2016.02.004.

Bridging the gap between DNA and RNA with Hox transcription factors

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Transcription factors (TFs) are key players in gene expression, acting on DNA regulatory sequences to coordinate morphogenetic programs. Beyond this DNA-centric viewpoint, several TFs interact with RNA and regulate alternative splicing, thus diversifying their gene-regulatory repertoire. Yet, the mechanisms by which TFs influence splicing and how TF-RNA function contributes to tissue morphogenesis remain elusive. We address this issue for the Drosophila Hox protein Ultrabithorax (Ubx), a central TF in metazoan development. By examining physical and functional interactions between wild-type and DNA-binding mutant proteins, we uncover a homodimerization mechanism employed by Ubx to regulate splicing, which relies on its ability to bind DNA and RNA. Moreover, we identify a key residue in the Ubx homeodomain that is critical for its RNA-binding ability. Overall, our work reveals the significant role of Ubx-RNA binding in muscle morphogenesis and Hox homeotic functions. We propose a refined model for Ubx splicing functions wherein TF-DNA/RNA binding and dimerization are essential for bridging transcription and splicing spatially. Our research highlights the key integrative function of TF-DNA and TF-RNA binding in orchestrating morphogenetic programs during animal development.

Translation elongation dynamics: an unbiased analysis of the ribosome pausing and collision.

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Translation elongation is a highly dynamic process in which ribosomes can be subject to drastic changes in decoding speed, leading to local ribosome pausing, stalling and collision events. Such ribosome collisions – where a fast upstream ribosome stacks onto a slower, downstream ribosome to form a disome, trisome or even higher-order collision – have been shown to occur in wild-type cells and organs even under regular growth conditions, where they likely represent transitory states of healthy protein biosynthesis1,2,3,4. In addition, the accumulation of collided ribosomes upon certain stresses and their ability to act as signaling platforms has revealed a surprising role in the cellular stress response and in cell fate decisions5,6. One of the main recently identified collision sensors is the kinase ZAK_ that auto-phosphorylates on collided ribosomes and triggers the MAP3K pathway via the phosphorylation of p38 and JNK, leading subsequently to cell death and apoptosis5,6. It remains a key question what distinguishes the two types of collisions, i.e. those that are benign and occur as a byproduct of normal translation, and the ‘toxic' collisions that elicit stress signaling. We hypothesize that the two collision states will differ in the recruitment or release of ribosome-associated proteins from the collision event, as well as in the locations of the collision sites on the transcriptome. In order to address these questions, we have generated ribo/disome profiling and proteomics datasets in mESCs under wild-type conditions or upon treatment with low doses of anisomycin, a translation elongation inhibitor previously described for its ability to induce ribosome collisions. We have now identified specific ribosome stalling patterns and several proteins that interact or are released in a collision-specific fashion. We are further validating our findings and studying the possible functional roles of these observations in regulating collision responses. 1. Meydan S & Guydosh NR (2020). Disome and Trisome Profiling Reveal Genome-wide Targets of Ribosome Quality Control. Molecular Cell, 79(4), 588–602.e6. 2. Zhao T, Chen YM, Li Y, Wang J, Chen S, Gao N & Qian W (2021) Disome-seqreveals widespread ribosome collisions that promote cotranslational protein folding. Genome Biology, 22(1), 16. 3. Han P, Shichino Y, Schneider-Poetsch T, Mito M, Hashimoto S, Udagawa T, Kohno K, Yoshida M, Mishima Y, Inada T, & Iwasaki S. (2020) Genome-wide Survey of Ribosome Collisions. Cell Reports, 31(5), 107610. 4. Arpat AB, Liechti A, De Matos M, Dreos R, Janich P & Gatfield D. (2020). Transcriptome-wide sites of collided ribosomes reveal principles of translational pausing. Genome Research, 30(7), 985–999. 5. Vind AC, Snieckute G, Blasius M, Tiedje C, Krogh N, Bekker-Jensen DB, Andersen KL, Nordgaard C, Tollenaere MAX, Lund AH, Olsen JV, Nielsen H, Bekker-Jensen S. ZAK_ Recognizes Stalled Ribosomes through Partially Redundant Sensor Domains. Mol Cell. 2020 May 21;78(4):700-713.e7 6. Wu CC, Peterson A, Zinshteyn B, Regot S, Green R. Ribosome Collisions Trigger General Stress Responses to Regulate Cell Fate. Cell. 2020 Jul 23;182(2):404-416.e14.

How the DEAD-box protein DDX3X unravels HIV-1 gRNA structure

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DEAD-Box helicases are enzymes that bind and remodel RNA and ribonucleoproteins. They are involved in almost every step of RNA metabolism. DEAD-Box helicases are thus major players of gene expression dysregulation and intracellular parasite invasion such as retroviruses. However, how they bind and remodel their RNA targets is still unclear. Among many implications in pathologies, the human DEAD-Box helicase DDX3X has been shown to be hijacked by HIV-1 at various steps including viral RNA export from the nucleus and translation initiation of the Gag polyprotein, but little is known about the way it interacts with the viral genomic RNA as well as the consequences of this interaction upon HIV-1 RNA structure. Here, we show that DDX3X binds to specific regions of HIV-1 5'-UTR resulting in the dissociation of HIV-1 RNA model dimers. This unprecedented enzymatic activity for a DEAD-box protein is achieved with a strong efficiency and in multiple turn-over conditions. By coupling the biochemical analysis of DDX3X enzymatic activity to the systematic probing of HIV-1 derived RNAs structure, we challenge both the accepted structural model of HIV-1 gRNA dimers as well as the dogma considering DEAD box proteins as rather promiscuous towards their RNA substrates.

Human coronaviruses: Various 5'UTRs for different levels of translation efficiency

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Translation in eukaryotes occurs through a linear scanning mechanism, where the 43S preinitiation complex, comprising the small ribosomal subunit associated with initiation factors, binds to the mRNA's 5' cap and scans the 5'UTR until it reaches the AUG codon. As a result, the length and structure of the 5'UTR play a crucial role in regulating translation by determining how many ribosomes can participate in protein synthesis. Upon infection of the host-cell, the SARS-CoV-2 virus expresses about 9 subgenomic and the genomic RNAs that are all translated to produce viral proteins needed for efficient replication. All these mRNAs are expressed through a complex transcriptional mechanism relying on cis-acting RNA regions in the viral genome. Consequently, all these viral mRNAs harbor a 5'UTR with a common and unique 75nt leader sequence at the 5' end, followed by a variable sequence in length and RNA structures specific to each viral RNA. As a result, each viral transcript exhibits translational characteristics that are determined by the composition of its own 5'UTR. In this study, we measured and compared the translational efficiency of all 10 viral transcripts within the SARS-CoV-2 genome. Our findings demonstrate that most of these transcripts are efficiently translated in all tested systems (in vitro and ex-vivo). Intriguingly, even the 5'UTR of the genomic RNA, which is the longest (230 nucleotides) and the most structured, displayed efficient translation. This observation is particularly remarkable because this 5'UTR also contains an upstream Open Reading Frame (uORF), typically a strong inhibitory signal for ribosomal scanning and translation. To gain a deeper understanding of the molecular factors that influence variations in translation efficiency among the diverse 5'UTRs of SARS-CoV-2, we conducted an extensive analysis specifically targeting the regulatory elements within each 5'UTR. Collectively, our findings provide valuable insights into the intricate mechanisms governing the synthesis of viral proteins.

Minor splicing as a regulator of neuronal transcriptome plasticity?

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Alternative splicing is a well-known process that allows fine regulation of isoform expression at key stages of tissue development (1). In brain, it ensures many processes from neural progenitor stock turnover to axon guidance (2). Recently, light has been shed on how widespread intron retention (IR) is. Among the stock of pre-spliced mRNAs that remains available in the cell, some retained introns are spliced in response to stimuli (like action potential) allowing mRNA translation and quick adaptation to the environment (3). Splicing is ensured by two large ribonucleoproteic complexes, the major and the minor spliceosomes. While the former is in charge of excising the vast majority of (major/U2) introns, the latter only processes ~850 minor/U12 introns, found in ~750 genes (4). Taking advantage of the study of very rare microcephalic osteodysplastic growth restriction genetic syndromes, caused by mutations in the U4atac snRNA minor spliceosome component (5,6), we analysed at the molecular level the role of minor splicing during neuronal differentiation. We introduced two patient recurrent point mutations in U4atac in induced pluripotent stem cells that we differentiated into neuronal progenitors and neurons, and then performed in-depth bulk RNAseq. As expected, we observed specific and strong (40 to 70% of introns) alterations of U12 intron splicing, which are mostly seen as IR. These IR are not associated to decreased transcript levels, suggesting that transcripts with U12 IR are not subjected to RNA decay. Most surprisingly, our dataset also revealed that some U2 introns (2-4%) are, at the opposite, better spliced in U4atac-mutated neurons compared to controls. We interpret this result as a reduction of physiological major IR in neurons, which may thus impair the neuronal plasticity of U4atac-mutated cells. However, the correlation between U12 intron retention and U2 intron “over-splicing” remains unknown.

Through a clinico-biological approach, we also highlighted strong candidate genes to explain the pathomechanisms in RNU4ATAC-related neurological disorders, by analysing the 158 minor and 2620 major intron-containing genes associated with mendelian neurodevelopmental disorders. 107 (67%) of the U12 morbid genes presented significant retention of minor intron, while 308 (12%) U2 morbid genes exhibited major splicing alterations.

Altogether, our results unveil an unsuspected role for the minor spliceosome in gene regulation that goes beyond the sole splicing of minor introns, and that could participate to the pathomechanisms of RNU4ATAC-opathies.

References: 1. Mazin PV, Khaitovich P, Cardoso-Moreira M, Kaessmann H. Alternative splicing during mammalian organ development. Nat Genet. 2021 Jun;53(6):925–34. 2. Zheng S. Alternative splicing programming of axon formation. WIREs RNA. 2020;11(4):e1585. 3. Mauger O, Lemoine F, Scheiffele P. Targeted Intron Retention and Excision for Rapid Gene Regulation in Response to Neuronal Activity. Neuron. 2016 Dec 21;92(6):1266–78. 4. Turunen JJ, Niemela EH, Verma B, Frilander MJ. The significant other: splicing by the minor spliceosome: Splicing by the minor spliceosome. Wiley Interdiscip Rev RNA. 2013 Jan;4(1):61–76. 5. Edery P, Marcaillou C, Sahbatou M, Labalme A, Chastang J, Touraine R, et al. Association of TALS Developmental Disorder with Defect in Minor Splicing Component U4atac snRNA. Science. 2011 Apr 8;332(6026):240–3. 6. He H, Liyanarachchi S, Akagi K, Nagy R, Li J, Dietrich RC, et al. Mutations in U4atac snRNA, a Component of the Minor Spliceosome, in the Developmental Disorder MOPD I. Science. 2011 Apr 8;332(6026):238–40.

Implementing and upgrading the Nanoseq pipeline for ONT long read analysis in a mouse model of muscular dystrophy type 1

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Third-generation sequencing technology produces long reads that can help us address new questions that are impossible to address with short-read sequencing. However, the analysis of this new type of data requires the development of appropriate informatics programs optimized for the handling of

long reads. Nanoseq1 is an existing nf-core pipeline for DNA, cDNA or direct RNA analysis of sequencing data produced by Oxford Nanopore Technology (ONT). Our team wanted to test and improve it with the most useful programs for our research, particularly for the analysis of transcriptome variations such as alternative splicing.

After a quality control step, Nanoseq aligns the reads on the genome using specific mapping tools (minimap2 or Graphmap2), builds a transcriptome (bambu, stringtie2) and analyses the differential expression of transcripts using DESeq2 and DEXSeq. For RNA data, nanoseq also uses the fast5 output format of ONT sequencing to detect RNA modifications such as m6a methylation (Nanopolish, Xpore, m6anet). After implementation, we added several new software and

commands. First, FLAIR2 to define transcript isoforms and analyse alternative splicing. Next, we

modified Nanopolish3 commands to estimate the polyA tail size of sequenced transcripts. Finally, we added some secondary options to filter and trim reads after basecalling, which were missing in the original pipeline.This pipeline will allow us to analyze direct RNA sequencing data and to detect and quantify the differential expression of transcripts between two conditions, using complementary FLAIR and stringtie2 outputs. Another objective is to visualize transcript isoforms and to detect differential splicing events with FLAIR. Finally, we will be able to compare the length of the poly- A tail of RNA isoforms that are produced in different conditions, depending on the used promoter or polyA site.

To test the pipeline, we carried out a pilot experiment to analyze RNA samples purified from astrocytes isolated from the brains of 2 types of mice: wild-type mice and the so-called DMSXL

mouse model, which simulates the DM1 phenotype4, with the aim of deciphering the brain dysfunction of this disease. One RNA sample from each condition (WT and DMSXL) was sequenced and base-called with Dorado to generate fastq files, the input format for the nanoseq pipeline. We will present our first results from this analysis which is still ongoing.

1. Nanoseq/CITATIONS.md at master · nf-core/nanoseq. (s. d.). GitHub. Consulté 25 septembre 2024, à l'adresse https://github.com/nf-core/nanoseq/blob/master/CITATIONS.md

2. Tang, A. D., Soulette, C. M., van Baren, M. J., Hart, K., Hrabeta-Robinson, E., Wu, C. J., & Brooks, A. N. (2020). Full-length transcript characterization of SF3B1 mutation in chronic lymphocytic leukemia reveals downregulation of retained introns. Nature Communications, 11(1), 1438. https://doi.org/10.1038/s41467-020-15171-6

3. Simpson, J. T., Workman, R. E., Zuzarte, P. C., David, M., Dursi, L. J., & Timp, W. (2017). Detecting DNA cytosine methylation using nanopore sequencing. Nature Methods, 14(4), 407-410. https://doi.org/10.1038/nmeth.4184

4. Dincã, D. M., Lallemant, L., González-Barriga, A., Cresto, N., Braz, S. O., Sicot, G., Pillet, L.-E., Polvèche, H., Magneron, P., Huguet-Lachon, A., Benyamine, H., Azotla-Vilchis, C. N., Agonizantes-Juárez, L. E., Tahraoui-Bories, J., Martinat, C., Hernández- Hernández, O., Auboeuf, D., Rouach, N., Bourgeois, C. F., ... Gomes-Pereira, M. (2022). Myotonic dystrophy RNA toxicity alters morphology, adhesion and migration of mouse and human astrocytes. Nature Communications, 13(1), 3841. https://doi.org/10.1038/s41467-022-31594-9

The interferon-sensitive gene 20 kDa (ISG20) induces nucleolar reorganization, regulates non-coding RNA expression, and restricts RNA viruses via nucleolar translocation

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The Interferon-sensitive gene 20 kDa protein (ISG20) is a 3'-to-5' RNA exonuclease that restricts the replication of a broad range of RNA viruses. While the main antiviral mechanism described in the literature indicates that ISG20 can directly degrade viral RNA thanks to its RNase activity, this is not observed homogeneously and alternative mechanisms have recently been proposeda (1a 7). Using Vesicular Stomatitis Virus (VSV) as a model for a highly replicative RNA virus, our laboratory has recently proposed that ISG20 could specifically inhibit the translation of viral RNAs without degrading them in a manner that required an intact RNase domain. Interestingly, we determined that this mechanism of translational control targeted also RNA generated from transiently transfected DNA, but was however unable to target RNA generated from an integrated DNA. As such we proposed that ISG20 could act as a translational safeguard and that it was able to distinguish self-RNA from non-self RNA (7).a According to these results, we formulated the hypothesis that ISG20 may degrade a cellular RNA, which in turn inhibits translation of viral RNAs. Using a combination of approaches that include microscopy as well as transcriptomics, we discovered that ISG20 rapidly translocates into the nucleolus in response to either viral infection or interferon stimulation and this translocation is required for its antiviral activity. At this location, ISG20 reorganizes the shape of nucleoli and promotes nucleolar fusion. Molecularly, this relocalization is accompanied by thea modulation of several small RNA classes and most notably of a subset of C/D-box small nucleolar RNAs (snoRNAs) as well as of the U6 snRNA, which are up- and down-regulated respectively. The changes in nucleolar shape and small RNA associated with ISG20 entry into the nucleolus suggest a possible link to explain the translation shutoff of viral RNA. These and other results aimed at understanding the functional relationship between ISG20, nucleolar functions and viral inhibition at translation will be presented.a This work received the support of the ANR, a oegrant number ANR-20-CE15-0025-01a ù a References 1. Liu Y, Nie H, Mao R, Mitra B, Cai D, Yan R, et al.a Interferon-inducible ribonuclease ISG20 inhibits hepatitis B virus replication through directly binding to the epsilon stem-loop structure of viral RNA. PLoS Pathog. avr 2017;13(4):e1006296. 2. Espert L, Degols G, Lin YL, Vincent T, Benkirane M, Mechti N. Interferon-induced exonuclease ISG20 exhibits an antiviral activity against human immunodeficiency virus type 1. J Gen Virol. aoÃ»t 2005;86(Pt 8):2221a ‘9.a 3. Espert L, Degols G, Gongora C, Blondel D, Williams BR, Silverman RH, et al. ISG20, a new interferon-induced RNase specific for single-stranded RNA, defines an alternative antiviral pathway against RNA genomic viruses. J Biol Chem. 2 mai 2003;278(18):16151a ‘8.a 4. Leong CR, Funami K, Oshiumi H, Mengao D, Takaki H, Matsumoto M, et al. Interferon-stimulated gene of 20 kDa protein (ISG20) degrades RNA of hepatitis B virus to impede the replication of HBV in vitro and in vivo. Oncotarget. 18 oct 2016;7(42):68179a ‘93.a 5. Stadler D, KÃ_chele M, Jones AN, Hess J, Urban C, Schneider J, et al. Interferon-induced degradation of the persistent hepatitis B virus cccDNA form depends on ISG20. EMBO Rep. 4 juin 2021;22(6):e49568.a 6. Weiss CM, Trobaugh DW, Sun C, Lucas TM, Diamond MS, Ryman KD, et al. The Interferon-Induced Exonuclease ISG20 Exerts Antiviral Activity through Upregulation of Type I Interferon Response Proteins. mSphere.a 19 sept 2018;3(5):e00209-18.a 7. Wu N, Nguyen XN, Wang L, Appourchaux R, Zhang C, Panthu B, et al.a The interferon stimulated gene 20 protein (ISG20) is an innate defense antiviral factor that discriminates self versus non-self translation.a PLoS Pathog. oct 2019;15(10):e1008093.a a

Interplay of m6A Methylation, and Post-transcriptional Regulation: The Role of ELAVL1 and hnRNPC in hepatocarcinoma cells.

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Hepatocellular carcinoma (HCC), the highly lethal form of primary liver cancer, is characterized by complex molecular alterations, including epigenetic and post-transcriptional modifications. One-carbon metabolism, a crucial pathway integrating folate and methionine cycles, generates metabolites such as S-adenosylmethionine (SAM) that serves as methyl donors for RNA methylation, particularly N6-methyladenosine (m6A). This dynamic and reversible RNA modification is a key regulator of gene expression in cancer cells. In HCC, m6A reader proteins (i.e. YTHDF2, ELAVL1 and hnRNPC) play pivotal roles in regulating mRNA splicing, stability and translation, influencing the oncogenic potential of tumor cells. This study explores the interplay between m6A methylation of one-carbon metabolism transcripts, and post-transcriptional regulation in HCC cells. MAT2A and MAT1A, which encode the two subunits of methionine adenosyl-transferase, are regulated positively by ELAVL1 and negatively by hnRNPD, respectively in HCC. For the first time, we have identified various RNA-binding proteins (RBPs) in HCC cells, including ELAVL1, hnRNPC, YTHDF2 and hnRNPD, which regulate many transcripts involved in one-carbon metabolism, such as MAT2A, MAT1A, MTR (which encodes methionine synthase), and other mRNAs, through RNA immunoprecipitation analysis. In parallel, we aim to explore the impact of m6A methylation on the regulation of these mRNAs. To this end, we analyzed in HCC cells the m6A methylation profiles of specific transcripts related to one-carbon metabolism. Notably, MTR and MAT2A exhibit higher methylation levels compared to MAT1A. Additionally, data from the m6A ATLAS database revealed interactions between these mRNAs and specific RBP (ELAVL1 and hnRNPC) at the same m6A sites. This suggests that a specific m6A methylation site may regulate the coordinated functions of these RBPs, influencing RNA maturation processes such as splicing, stability, and transport, a study currently underway.

Deciphering the translation dynamics of SARS-CoV2 RNAs

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Severe acute respiratory syndrome coronavirus 2 (SC2), responsible for the current COVID-19 pandemic, represents a serious global public health problem. SC2 is a positive RNA-enveloped virus of the Betacoronaviridae family, it primarily infects human lung cells and can cause severe respiratory disease. Advances in fundamental research into its genomic RNA localization, translation and packaging are needed to develop new therapies and vaccines.

During the infectious cycle of SC2, the transcription of the genomic RNA (gRNA) occurs into Double-Membrane Vesicles (DMVs). Following transcription, the gRNA exits these vesicles and will be packaged during the assembly of viral particles. Additionally, SC2 as well as other Betacoronaviridae employs a discontinuous transcription mechanism, generating subgenomic RNAs (sgRNAs) encoding both structural and accessory proteins. This complex process results in the production of a pool of sgRNAs, each containing a common leader sequence and a variable part in their 5' untranslated regions (UTRs).

SC2 produces up to 10 canonical sgRNAs in addition to its gRNA. A critical component in the transcription process of SC2 is the Transcription-Regulating Sequence (TRS). Preceding each viral gene, TRS-Body (TRS-B) comprises a core sequence and variable flanking sequences. Positioned at the 5_ end of the genome, the TRS of the leader (TRS-L) contains the core sequence and is exposed. Discontinuous transcription occurs during the negative-strand RNA synthesis when TRS-B copy hybridizes with TRS-L. Due to the variability of TRS-B, each sgRNA, as well as the gRNA, will possess a distinct 5' UTR and/or a distinct Kozak context. This diversity potentially results in differences in their translation dynamics.

The project aims to quantitatively characterize the molecular and cellular mechanisms of vRNAs translation in different human cell lines. The focus is mainly on the roles of the different 5'UTRs on the translation of the subgenomic and genomic RNAs to understand the spatio-temporal coordination of viral protein production. Real-time monitoring of vRNA localization and translation kinetics in living cells will be carried out using live imaging of nascent peptide systems (SunTag/ALFA-array)1-4. Advanced experiments, such as fluorescence correlation spectroscopy and single particle tracking, will be used to extract translation kinetics1,4. This global project aims to improve understanding of the temporal replication of SARS-CoV-2, from mRNA translation of structural viral proteins to viral particle biogenesis, paving the way for new advances in the fight against this pandemic.

1. Dufourt, J. et al. Imaging translation dynamics in live embryos reveals spatial heterogeneities. Science (2021).
2. Bellec, M. et al. Boosting the toolbox for live imaging of translation. RNA (2024).
3. Yan, X. et al. Dynamics of Translation of Single mRNA Molecules In Vivo. Cell (2016).
4. Morisaki, T. et al. Real-time quantification of single RNA translation dynamics in living cells. Science (2016).

Regulation of CagA oncoprotein expression in the gastric pathogen Helicobacter pylori.

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Helicobacter pylori colonizes the stomach of half the world's population. This Gram-negative bacterium is responsible for gastric cancers such as adenocarcinomas and MALT-type lymphomas. With an estimated 810,000 new cases in 2018, it is the leading infectious cause of cancer. The main virulence factor associated with a high risk of gastric cancer is the cag pathogenicity island (cag-PAI). This 40 kb genomic region encodes a type IV secretion system responsible for translocating the CagA virulence factor into host cells. Delivered CagA interferes with multiple host signalling pathways promoting gastric carcinogenesis. While in most H. pylori strains (type A) the cagA gene belongs to the cag-PAI, a subset of strains (type B) underwent a genomic rearrangement leading to the separation of cagA from the cag-PAI. This phenomenon introduced heterogeneity in the sequence and copy number of the cagA gene. Although the impact of this genetic organization on bacterial pathogenicity remains unknown, recent studies suggest that it affects the expression of the CagA protein. While both type A and type B H. pylori strains share a common cagA promoter, our results identified an additional promoter in type B strains which is located 100 nucleotides upstream of the common promoter. Moreover, type B strains also possess a promoter on the opposite cagA strand leading to the synthesis of a small antisense RNA. Here we analyse the contribution of these promoters to CagA protein expression in a type B strain. We show that although P1 is stronger than P2, the expression of CagA protein is mainly driven by P2. Instead, transcription from P1 slightly inhibits P2 and leads to the synthesis of a 200 nt small non-coding RNA. Moreover, our findings revealed that the copy number of cagA gene varies amongst different isolates of B128 type B strain.In conclusion, we have identified a complex transcriptional activity at the cagA locus which may regulate CagA protein expression, allowing H. pylori to control inflammation.

Coupling mRNA and peptide degradation pathways into the ribosome-associated quality control

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Accumulation of impaired proteins poses a critical issue for all cells, and elaborate quality control systems have evolved to minimize their effects. In particular, cells have developed failsafe mechanisms to cope with deleterious ribosomal stalls that result from damaged mRNA. Though temporary ribosome stalls can have biological functions, like co-translational protein folding or targeting, persistent stalls are generally indicative of a stress, leading to ribosomal collisions, promoting degradation of the nascent peptide (NP) and triggering mRNA decay. Recognition of ribosomal collisions involves factors and mechanisms that are highly conserved in eukaryotes, as exemplified in the S. cerevisiae model. Thus, ribosome collisions form a particular conformation recognized by a conserved ubiquitin ligase, Hel2. Specific ribosomal proteins are then ubiquitinated and associate with the Ribosome Quality Triggering (RQT) complex, which disassembles the colliding ribosomes and generates a 60S subunit still attached to a peptidyl RNA. Then, the Ribosome-mediated Quality Control (RQC) pathway can interact with this particular 60S subunit, and triggers the degradation of the arrested product by the proteasome system. It is proposed that this quality control is coupled to mRNA decay processes that target problematic mRNAs and prevent ribosomes from continuously producing aberrant peptides, potentially toxic to the cell. It is now admitted that problematic mRNAs (stalling within coding sequence) are mostly degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. How all these processes are coordinated has not been clarified. Nor has the interconnection of these processes been elucidated, if it exists at all. To investigate whether mRNA and peptide degradation are coupled, we wondered whether mRNA decay factors are able to affect RQT and/or RQC processes. We thus designed different mRNA reporters containing rare codons, and analyzed the impact of a set of mRNA decay mutants on aberrant peptide production. The tricky part of this approach is that a greater stabilization of faulty mRNAs may simply result, in a passive mode, in the accumulation of aberrant peptides. We have therefore designed our mRNA reporters to limit this issue and we propose that PAT1 deletion, or specific mutations affecting Pat1, may disrupt the implementation of a correct quality control by RQT and/or RQC complexes. As no similar effect was observed for other mutants of decapping, we propose that Pat1 is a promising candidate interconnecting all these quality control processes via mechanisms and interactions to be deciphered.

Investigating RNA-Protein Interactions Using the Doped-SELEX Method

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RNA molecules, along with other oligonucleotides, can adopt complex three-dimensional structures that enable them to interact with a wide array of targets, including small molecules and proteins. Understanding the structural bases of these interactions is crucial for both fundamental biology and the development of RNA-based therapeutics. To explore these interactions in detail and to select nucleic acid structures with enhanced affinity for their targets, we employ a technique known as Doped-SELEX. This method involves generating a library from an initial oligonucleotide sequence, introducing random mutations across all positions. This mutated library is then incubated with the target of interest, allowing for the selection of sequences that bind most effectively. The bound sequences are subsequently analyzed using high-throughput sequencing (HTS). By examining millions of variants derived from the original sequence, Doped-SELEX reveals positions within the oligonucleotide that do not tolerate substitutions, indicating their critical role in maintaining interaction specificity and stability. Furthermore, this approach can uncover mutations that potentially enhance the binding affinity, offering insights into how RNA-protein interactions can be optimized. The Doped-SELEX technique thus serves as a powerful tool for dissecting the intricate details of RNA-protein interactions, facilitating the identification of key nucleotide positions that govern these interactions and enabling the rational design of RNA molecules with improved functional properties.

Fr: Analyse fonctionnelle du facteur d'epissage CAPERi±/RBM39, une cible de molecules antitumorales / En: Functional analysis of CAPERi±/RBM39 splicing factor, a target of antitumor molecules

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CAPER_ also known as RBM39 is a transcription and splicing factor which belongs to the RNA binding proteins (RBP) family. CAPER_ contributes to transcription regulation [1] and alternative RNA splicing [2]. It is also involved in tumorigenesis of some cancers such as osteosarcoma [3] and neuroblastoma [4], particularly through modulating splicing of target genes. Recently, studies have demonstrated that CAPER_ is an unexpected target of Indisulam, an aryl sulfonamide molecule with anti-tumoral properties [5]. Such discovery could be the key of a promising therapeutic approach against CAPER_-related cancers. We tried to unravel the mechanism of CAPER_ recruitment in the spliceosome and identify CAPER_ interactome by the means of co-immunoprecipitation, GST-pulldown and immunostaining experiments. Afterwards, we used splicing reporters after CAPER_ shRNA depletion or overexpression to determine the effects of CAPER_ on splicing. CAPER_ is recruited in the spliceosome complex thanks to its interactions with U2AF65 and SF3b1. CAPER_ UHM domain interacts with SF3b1 ULM domain [6], while CAPER_ U2AF65 interaction relies on their RS domains. Such interaction could lead to liquid-liquid phase separation (LLPS), with droplets enriched with CAPER_ and U2AF65 proteins [7]. LLPS drives the formation of nuclear dynamic membraneless bodies called nuclear speckles, which concentrate RBPs and RNAs. In fact, CAPER_ can remodel the composition of these speckles by recruiting its partners through heterotypic interactions depending on RS domains. Concerning splicing, even though we could not completely unravel how CAPER_ recognizes its RNA targets, the strength of 3' splicing site seems to play a crucial role. [1] D.-J. Jung, S.-Y. Na, D. S. Na, et J. W. Lee, « Molecular Cloning and Characterization of CAPER, a Novel Coactivator of Activating Protein-1 and Estrogen Receptors * », J. Biol. Chem., vol. 277, no 2, p. 1229_1234, janv. 2002, doi: 10.1074/jbc.M110417200. [2] S. Mai, X. Qu, P. Li, Q. Ma, C. Cao, et X. Liu, « Global regulation of alternative RNA splicing by the SR-rich protein RBM39 », Biochim. Biophys. Acta BBA - Gene Regul. Mech., vol. 1859, no 8, p. 1014_1024, aout 2016, doi: 10.1016/j.bbagrm.2016.06.007. [3] G. Huang, Z. Zhou, H. Wang, et E. S. Kleinerman, « CAPER-_ alternative splicing regulates the expression of vascular endothelial growth factor___ in Ewing sarcoma cells », Cancer, vol. 118, no 8, p. 2106_2116, avr. 2012, doi: 10.1002/cncr.26488. [4] S. Singh et al., « Targeting the spliceosome through RBM39 degradation results in exceptional responses in high-risk neuroblastoma models », Sci. Adv., vol. 7, no 47, p. eabj5405, nov. 2021, doi: 10.1126/sciadv.abj5405. [5] T. Han et al., « Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15 », Science, vol. 356, no 6336, p. eaal3755, avr. 2017, doi: 10.1126/science.aal3755. [6] S. Loerch, A. Maucuer, V. Manceau, M. R. Green, et C. L. Kielkopf, « Cancer-relevant Splicing Factor CAPER_ Engages the Essential Splicing Factor SF3b155 in a Specific Ternary Complex », J. Biol. Chem., vol. 289, no 25, p. 17325_17337, juin 2014, doi: 10.1074/jbc.M114.558825. [7] M. Tari, V. Manceau, J. De Matha Salone, A. Kobayashi, D. Pastre, et A. Maucuer, « U2 AF 65 assemblies drive sequence_specific splice site recognition », EMBO Rep., vol. 20, no 8, p. e47604, aout 2019, doi: 10.15252/embr.201847604.

Study of alternative splicing during erythropoiesis in i_-hemoglobinopathies

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β-hemoglobinopathies are a group of inherited blood disorders characterized by either quantitative or qualitative defects in β-globin chain synthesis, which affect the expression of adult hemoglobin (HbA). Throughout human development, there is a switch in the type of hemoglobin (Hb) produced. Fetal hemoglobin (HbF), which is composed of γ-globin chains, is replaced by β-globin chains at birth. It is known that the continued production of HbF γ- chains in adult patients can attenuate the clinical severity of β-globin defects. Hb switching is the result of tightly regulated changes in the transcriptome, yet the precise molecular mechanisms responsible for the developmental repression of γ-chains in adult red blood cells (RBCs) remain incompletely characterized. It has been proposed that post-transcriptional regulation of gene expression plays a key role in this process. Splicing modulation is emerging as an attractive approach to alleviate transcriptional repression of the fetal hemoglobin γ- chains. This project aims to conduct a comprehensive analysis of the splicing regulations that occur during both adult and fetal erythropoiesis in red blood cells (RBCs). We employ long- read sequencing to conduct a differential analysis of transcript diversity in order to identify a regulatory network that contributes to the repression of fetal Hb γ-chains. Characterizing this network could then serve as a foundation for developing new therapeutic strategies aimed at enhancing γ-chain expression in the erythroid cells of adult patients.

Structure of the Nmd4-Upf1 complex supports conservation of the nonsense-mediated mRNA decay pathway between yeast and humans

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The nonsense-mediated mRNA decay (NMD) pathway clears eukaryotic cells of mRNAs containing premature termination codons (PTC) or normal stop codons located in specific contexts. It therefore plays an important role in gene expression regulation. The precise molecular mechanism of the NMD pathway has long been considered to differ substantially from yeast to metazoa, despite the involvement of universally conserved factors such as the central ATP-dependent RNA-helicase Upf1. Recently, Upf1 protein was shown to be part of two distinct and mutually exclusive complexes in the yeast S. cerevisiae : the Upf1-2/3 complex and the Upf1-decapping complex. The latter encompasses Upf1, the Dcp1, Dcp2 and Edc3 decapping factors as well as two largely uncharacterized proteins, Nmd4 and Ebs1. These two additional yeast NMD co-factors show similarities with metazoan SMG6 and SMG5/7, both at the level of their sequence and in term of their interactions with Upf1. We will describe the crystal structure of the yeast Upf1 bound to Nmd4, show that this latter stimulates Upf1 ATPase activity and that this interaction contributes to the elimination of NMD substrates. Our study demonstrates that a region of Nmd4 critical for the interaction with Upf1 in yeast is conserved in the metazoan SMG6 protein, another major NMD factor. This conserved region is involved in the interaction of SMG6 with UPF1, and mutations in this region affect the levels of endogenous human NMD substrates. Our results support the universal conservation of the NMD mechanism in eukaryotes.

Deciphering the Interplay Between Ribosome Hibernation Factors and RNA Modifications in Vibrio cholerae Antibiotic Resistance

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tRNA and rRNA modifications play crucial roles in bacterial biology, regulating various cellular processes such as mRNA translation and ribosome assembly. A recent study from our laboratory highlighted the significance of these modifications in Vibrio cholerae, the bacterium responsible for cholera1. Our results revealed that the presence or absence of specific modifications can either confer advantages or disadvantages in the presence of certain antibiotics at subinhibitory concentrations (subMIC). Building on these findings, (1) we aimed to exhaustively map these RNA modifications in V. cholerae using a variety of detection methods. By comparing our results with E. coli, where the full range of tRNA and rRNA modifications is already well-characterized, we identified unique modifications specific to V. cholerae. (2) We focused particularly on a modification carried out by RluB, a pseudouridine synthase that catalyzes the formation of pseudouridine at position 2588 of the 23S rRNA in V. cholerae. A rluB mutant exhibited an advantage when exposed to subinhibitory concentrations (subMIC) of tobramycin, an antibiotic that targets the 30S ribosomal subunit. Preliminary data suggest that ribosome hibernation factors, especially the protein HpF2, may play a role in this phenotype. More generally, our research suggests an interplay between rRNA modifications and hibernation factors linked to antibiotic tolerance and resistance.

1. A, B. et al. Nonessential tRNA and rRNA modifications impact the bacterial response to sub-MIC antibiotic stress. microLife 3, (2022).

2. Sato, A. et al. Solution structure of the E. coli ribosome hibernation promoting factor HPF: Implications for the relationship between structure and function. Biochem. Biophys. Res. Commun. 389, 580–585 (2009).

Structural and functional characterization of Nakaseomyces glabrata Nudix hydrolase Npy1 involved in mRNA decapping

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Nakaseomyces glabrata, a closely related species to the Candida clade, is a significant opportunistic pathogen, especially in immunocompromised individuals, where systemic infections result in a high mortality rate of 40-60%. The increasing incidence of systemic candidiasis, along with rising resistance to conventional antifungal treatments like azoles, underscores the urgent need for novel therapeutic strategies. One potential target is the Npy1 enzyme, a NADH pyrophosphatase from the Nudix hydrolase superfamily. Npy1, characterized in Saccharomyces cerevisiae and N. glabrata, hydrolyzes NAD+ and NADH in the presence of Mn2+, producing NMNH and AMP. It's also involved in the process of mRNA NAD+ decapping. The enzyme's activity relies on a conserved motif and the presence of divalent cations. The homologous protein in N. glabrata shares 54% identity with the S. cerevisiae Npy1 and retains key structural features, including a peroxisomal targeting sequence. Recombinant Npy1 from N. glabrata was expressed and purified, showing catalytic activity toward NAD+ and NADH, confirming its functional role in NAD metabolism. Additionally, an in silico study revealed that NAD analogs could bind effectively to the enzyme's catalytic site, adopting orientations similar to its natural substrates. These findings suggest that Npy1 could be a promising target for the development of new antifungal therapies, particularly against N. glabrata infections resistant to existing treatments.

Role of Rho-dependent transcription termination in the biogenesis of small regulatory RNAs in the human pathogen Helicobacter pylori

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Transcription termination plays a key role in gene expression in all organisms because it defines RNA 3' ends, recycles RNA polymerase, and prevents improper expression of downstream genes. Bacteria use two pathways to terminate transcription: Intrinsic (factor-independent) termination, which is triggered by RNA hairpin structures followed by a stretch of uridines in the nascent transcript and Rho-dependent transcription termination (RDTT), which requires the hexameric translocase/helicase Rho. Whereas these mechanisms have been well studied in the Escherichia coli model organism, much less is known about these processes in other bacteria. Here, we highlight the prominent role of RDTT in the human gastric pathogen Helicobacter pylori. This Epsilon-proteobacterium persistently colonizes the stomach of half of the human population, causing severe pathologies such as gastric cancer. We have shown that intrinsic terminator-like structures present at the 3' ends of some H. pylori regulatory small RNAs (sRNA) do not stop transcription efficiently. Further, inhibition of Rho with bicyclomycin induces transcriptional readthrough of many sRNAs, the amount of some being also greatly reduced. We also showed, both in vivo and in vitro, that regions downstream of the mature sRNA 3' ends are C>G-biased, typical of Rho binding sites (Rut sites), and are necessary for RDTT. Finally, we also discovered that Rho is essential in H. pylori. Altogether, our results reveal a major role for Rho in the biogenesis of small regulatory RNAs in H. pylori. Our next objective is to understand how RDTT contributes to the virulence and to the adaptive mechanisms of this major human pathogen.

Inhibition of rRNA maturation in triple negative breast cancer

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Ribosome biogenesis involves multiple steps and is regulated by over 200 factors. Initially, a 47S pre-ribosomal RNA (pre-rRNA) is synthesized in the nucleolus by RNA polymerase I (Pol I), and 5S rRNA by RNA polymerase III in cytosol. The pre-rRNA undergoes maturation, including cleavages and chemical modifications, and assembles with ribosomal proteins into subunits, which are then exported to the cytosol to assemble as mature ribosomes. Cancer cells have been shown to produce more ribosomes than normal cells, which allows them to preferentially translate specific mRNAs that drive their proliferation. Consequently, cancer cells are highly dependent on increased ribosome biogenesis and are sensitive to its inhibition. Recent studies on Pol I inhibitors, such as CX-5461 and BMH-21, which target the initial step of ribosome biogenesis, have provided proof of concept that inhibiting ribosome biogenesis can induce antiproliferative and cytotoxic effects in cancer cells, while sparing healthy cells, in various cancer types. However, despite their effectiveness, current Pol I inhibitors are transcription inhibitors that were reported to cause genotoxic effects. Ribosome biogenesis rely on many other factors essential for ribosome production that may serve as promising therapeutic targets. One such factor involved in rRNA maturation is Fibrillarin (FBL). FBL is responsible for pre-rRNA cleavage and the post-transcriptional 2'-O-methylation of rRNA. We previously showed that inhibiting FBL impairs pre-rRNA cleavage and alters the 2'-O-methylation pattern of rRNA, affecting the translation of certain mRNAs [1]. In this context, we investigated FBL inhibition in triple-negative breast cancer (TNBC), a type of cancer with currently limited therapeutic options. FBL is overexpressed breast in cancers, particularly in the TNBC subtype. In our study, we determined that the knockdown of FBL induces a halt in colony formation and cell growth in vitro. In vivo, mouse xenograft demonstrated the antitumorigenic effect of FBL knockdown. Additionally, we show that the knockdown of FBL induces cell cycle arrest in a p53-mutated cell type. Thus, targeting the pre-rRNA maturation step emerges as a potential new therapeutic approach downstream of rRNA synthesis. [1] : Erales, Jenny, Virginie Marchand, Baptiste Panthu, Sandra Gillot, Stephane Belin, Sandra E. Ghayad, Maxime Garcia, et al. 2017. « Evidence for rRNA 2'-O-Methylation Plasticity: Control of Intrinsic Translational Capabilities of Human Ribosomes ». Proc Natl Acad Sci USA 114 (49): 12934_39. https://doi.org/10.1073/pnas.1707674114.

Regulation of nuclear speckle protein SON as a crucial mechanism for proper neurodevelopment

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Brain development is a complex process, requiring an intricate interplay between various biological processes. In recent years, the importance of alternative splicing in brain development has been highlighted, yet how alternative splicing is tuned to the dynamic alterations that neurons undergo during maturation, remains to be elucidated. The autism spectrum disorder-related kinase NUAK1 was identified as a protein crucial for the formation of functional neuronal networks, regulating axon length and branching in mouse cortical neurons. Here, we propose evidence that NUAK1 is a critical regulator of splicing in developing cortical neurons. Interestingly, we found that NUAK1 is able to phosphorylate the nuclear speckle protein SON and exerts its effects on splicing at least in part through SON, which is linked to a rare (neuro)developmental syndrome called ZTTK. shRNA-mediated knockdown of SON in murine cortical neurons mimics the effect of NUAK1 loss on axon length and branching, suggesting that NUAK1 and SON operate in the same pathway to regulate neuronal development. Indeed, deep-sequencing of RNA of neurons in which either NUAK1 or SON was knocked down showed that there is a significant overlap in aberrant splicing events affecting basic neuronal processes, such as axon growth and branching, but also dendrite formation and synaptic processes. In summary, we propose the discovery of a novel pathway involving NUAK1 and SON, which regulates splicing events in neurons that ultimately impact neuronal development and function. This work is funded by Marie-Sklodowska Curie Actions and Fondation de Recherche Medicale.

The 3'-5' RNA Degradation Machinery in Hyperthermophile Archaea: Composition, Localization, and Function of the RNA Exosome

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Our study model are the hyperthermophile archaea Thermococcus barophilus and Pyrococcus abyssi from Thermococcales order. Their ability to grow at temperatures above 100°C provides unique opportunity to address the stability of RNA, DNA and proteins at extremely high temperatures. Archaea are ubiquitous in all ecosystems (oceans, soil, microbiota) but serve as exemplary models for the study of life in extreme conditions. Their unique, intermediate status between bacteria and eukaryotes offers insights into the evolution of both molecular and genetic processes and central metabolic pathways. We have identified key players of RNA degradation machinery in Thermococcales: the RNA exoribonucleases aRNaseJ (5'-3') and the RNA Exosome (3'-5'), as well as RNA Helicases - ASH-Ski2 & Lhr2. The objective of our research is to gain insight into the mechanisms by which these factors are specifically recruited to regulate the RNA life cycle at the post-transcriptional level. The network of molecular interactions established in Thermococcales by affinity pull-down experiments suggests the existence of interactions between 5' and 3' degradation machineries, as well as with the ribosome and helicases. Our recent research has focused on the comprehensive characterization of the RNA exosomes. The RNA exosome in archaea is constituted by nine protein subunits that assemble to form a barrel-like structure, with a cap at its apex. The barrel is composed of a trimer of Rrp41/Rrp42 dimers. The phosphorolytic activity of the complex is carried out by the three Rrp41 subunits. The cap is comprised of a trimer of Rrp4 and Csl4, the in vivo stoichiometry of which is currently unknown. This trimeric complex serves to facilitate the recognition of RNA substrates and their subsequent insertion into the lumen of the catalytic barrel, where the RNA degradation occurs. This 3'-5' degradation machinery is orthologous to nuclear and cytoplasmic RNA exosomes in eukaryotes. We aim at characterizing the RNA exosome composition in vivo, its protein partners and substrates. In this purpose, we have performed co-immunoprecipitations of the RNA exosome of T. barophilus followed by LC/MS analysis. All exosome subunits and the exosome's known in vitro partners, ASH-Ski2 and aRNaseJ, were successfully recovered. LC/MS analysis identified over 30 proteins that were enriched in the sample, among which were SMAP1 and Nop5. In order to characterize the different variants of exosome in vivo, we have reconstituted in vitro the RNA exosome with two different homogeneous caps (Rrp4 and Csl4). We have obtained cryo-electron microscopy (Cryo-EM) images of RNA exosomes at 3.4A resolution. These models serve as a basis for the subsequent in vivo characterization of the RNA exosome variants. We will present our preliminary data on in vivo RNA exosomes by cryo-EM.

Effects of phosphorylation on RISC activity

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MicroRNAs (miRNAs) are short, non-coding RNAs that associate with Argonaute (AGO) proteins to 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. miRNAs tether AGO1–4 proteins to their targets, triggering mRNA degradation or repressing translation. miRNAs play crucial roles in development and homeostasis. Consequently, dysregulation of miRNA is often linked to various diseases. AGO proteins may be subject to post-translational modifications, which occur following cell- or state-specific stimuli and may result in altered protein stability, cellular localization, and ability to bind miRNAs and to silence gene expression of target RNAs. Nevertheless, the impact of most post-translational modifications and their molecular mechanisms await exploration. We have begun characterizing how phosphorylation of specific amino acid residues modulates RISC activity. Our data should unravel basic mechanisms of modular layer of post-transcriptional control formed by miRNAs, which enables generating complex cellular responses to environmental challenges and pathological conditions.

Translation control or not, genetic tools for qualitative and quantitative exploration of gene regulation

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From transcription to protein synthesis, gene regulation is mediated by a plethora of effectors that can act in trans, such as transcription factors or regulatory RNAs, or in cis, such as riboswitches. Thanks to the improvement of global approaches, in particular transcriptomic and proteomic methods, our knowledge about regulatory circuits is rapidly increasing in most organisms. In contrast, the mechanistic elucidation of such circuits, especially of the precise step at which the controls take place, remains limited. This highlights a need for convenient tools to decipher the mode of action of regulatory elements. In this work, we designed a set of highly modular reporter constructs to quantitatively but also qualitatively analyze gene expression in the model bacterium Escherichia coli. These tools allow the expression of one or two fluorescent protein(s) under the control of ectopic transcriptional and translational signals. The locus carrying these constructs can be easily manipulated using CRISPR/Cas-assisted recombineering that relies on optimized guide RNAs, an inducible Streptococcus pyogenes Cas9 nuclease and the lambda red recombineering system. Furthermore, we optimized a library of counter-selectable plasmid borne gRNAs allowing to replace iteratively each building block of the reporter system in a single step, thereby ensuring the high modularity of these constructs. As a proof of concept, we used this approach to co-express two independently translated fluorescent proteins to qualitatively assess the step impacted by a regulatory element. More specifically, we show that this system allows to distinguish between purely translational regulation from other post-transcriptional regulatory events such as RNA degradation. In summary, by combining CRISPR/Cas-assisted recombineering and dual-fluorescent reporter systems we provide ready-to-use and modular genetic tools to decipher regulatory circuits in bacteria.

Role of the Long Non-Coding RNA ANRIL in Chromatin Landscape Regulation and its potential Association with Disease

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Long non-coding RNAs (lncRNAs), non-protein-coding transcripts longer than 200 nucleotides, have emerged as key regulators of gene expression in human, acting in both the cytoplasm and the nucleus. Nuclear lncRNAs can associate with the genome to recruit epigenetic complexes, which in turn mediate chromatin remodeling and influence gene expression. Thus, dysregulation of lncRNAs can affect these processes, promoting diseases like cancer by modulating cell proliferation, apoptosis, and differentiation for instance. One such lncRNA is ANRIL (Antisense Noncoding RNA in the INK4 Locus), transcribed from the 9p21 locus, which is likely to modulate gene expression by recruiting Polycomb Group (PcG) proteins, such as Polycomb Repressive Complexe 2 (PRC2) responsible for depositing the repressive histone modifications H3K27me3. ANRIL upregulation is frequently associated with various cancers and poor prognosis. However, the precise roles of ANRIL in gene silencing and tumor promotion are still not fully understood. We investigated the role of Exon 21 of ANRIL in modulating the chromatin landscape. Our findings indicate that this 3'-terminal exon plays a significant role in regulating ANRIL's activities. We observed drastic alterations in the chromatin landscape of H3K27me3 and H3K9me3 in cells lacking Exon 21 (HEK293-D21). Notably, we identified 24 domains that exhibited reduced H3K27me3 levels alongside increased H3K9me3 levels in the HEK293-D21 cell line. These findings suggest that Exon 21 promotes ANRIL-related PRC2 activities, thereby limiting H3K9me3 deposition. Furthermore, our data indicate that this mutually exclusive relationship likely influences the organization of Lamina-Associated Domains (LADs) and modulates the expression of genes involved in the senescence pathway. Thus, our study highlights the potential involvement of Exon 21 in ANRIL-mediated regulation of the nuclear lamina, which may, in turn, influence genome architecture and gene expression, potentially contributing to aberrant cell programming.

The dual life of nucleolar intrinsically disordered lysine-rich domains: from rRNA modification to nucleolar compaction

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Intrinsically disordered regions (IDRs) are highly enriched in the nucleolar proteome but their physiological role in ribosome assembly remains poorly understood. Our study reveals the functional plasticity of extremely abundant nucleolar lysine-rich IDRs associated with small nucleolar ribonucleoprotein particles (snoRNPs) from protist to mammalian cells. We show in Saccharomyces cerevisiae that the electrostatic properties of this lysine-rich IDR, the KKE/D domain, is required to promote SnoRNP accumulation to the vicinity of nascent rRNAs facilitating its modification. Under growth conditions reducing pre-rRNA synthesis and increasing the pool of latent snoRNPs, KKE/D domains act as a divalent ligand essential to promote condensation of Glycine-Arginine rich proteins associated with SnoRNP, but also nucleolar sequestration of a specific set of key early-acting ribosome biogenesis factors including RNA polymerase I. We propose that such mechanisms represent an ancestral eukaryotic regulatory system in which the self-interaction properties of latent SnoRNPs continuously coordinate nucleolar morphology with rRNA production level.

Critical cis-parameters influence STructure Assisted RNA Translation (START) initiation on non-AUG codons in eukaryotes

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In eukaryotes, translation initiation is a highly regulated process, which combines cis-regulatory sequences located on the messenger RNA along with trans-acting factors like eukaryotic initiation factors (eIF). One critical step of translation initiation is the start codon recognition by the scanning 43S particle, which leads to ribosome assembly and protein synthesis. In this study, we investigated the involvement of secondary structures downstream the initiation codon in the so-called START (Structure-Assisted RNA translation) mechanism on AUG and non-AUG translation initiation. The results demonstrate that downstream secondary structures can efficiently promote non-AUG translation initiation if they are sufficiently stable to stall a scanning 43S particle and if they are located at an optimal distance from non-AUG codons to stabilize the codon-anticodon base pairing in the P site. The required stability of the downstream structure for efficient translation initiation varies in distinct cell types. We extended this study to genome-wide analysis of functionally characterized alternative translation initiation sites in Homo sapiens. This analysis revealed that about 25% of these sites have an optimally located downstream secondary structure of adequate stability which could elicit START, regardless of the start codon. We validated the impact of these structures on translation initiation for several selected uORFs. - START: STructure-Assisted RNA Translation. Eriani G & Martin F (2018) RNA Biol 15, 1250-1253. - How Many Messenger RNAs Can Be Translated by the START Mechanism? Despons L & Martin F (2020) Int J Mol Sci 21, 8373. - Critical cis-parameters influence STructure assisted RNA translation (START) initiation on non-AUG codons in eukaryotes. Tidu A, Alghoul F, Despons L, Eriani G, Martin F. (2024) NAR Genom Bioinform 6: lqae065.

Reprogramming of BCL11A transcriptome to stimulate fetal i_-globin in i_-hemoglobinopathies

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β-hemoglobinopathies are the world’s most prevalent genetic disorders, resulting in ge- netic anemias caused by the absent, reduced, or abnormal synthesis of the β-chain of adult hemoglobin. To mitigate the clinical severity of these disorders, it has been demonstrated that the persistent production of the fetal γ-globin chain into adulthood can be an effec- tive therapeutic strategy. Interestingly, the transcription factor BCL11A, a critical repressor of fetal γ-globin expression in adulthood, is tightly regulated by alternative splicing and polyadenylation. This regulation results in the expression of various protein isoforms, not all of which are capable of repressing fetal γ-globin. We have launched a project aimed at reprogramming the transcriptome of BCL11A using antisense oligonucleotides (ASOs) to activate the expression of fetal globin during adult erythropoiesis. Using targeted long-read sequencing with Oxford Nanopore technology, we have uncovered a significantly more com- plex pattern of BCL11A transcript expression in both adult and fetal erythropoiesis. By integrating these techniques, we aim to identify cis-regulatory RNA sequences in BCL11A and use ASOs to influence γ-globin expression.

Challenges in RNA modification mapping by deep sequencing

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RNA modifications found in almost all types of cellular RNAs are now recognized as key players of RNA metabolism, affecting all steps of RNA lifea : processing/maturation, folding, recognition by cognate proteins and RNP assembly, trafficking, translation and degradation. Numerous methods are now available for RNA modification mapping in a subset of stable RNA species or transcriptome-wide. Despite substantial efforts for already >10 years in the epitranscriptome field, the consensus map of RNA modifications is only achieved for a few model living species and mostly only for stable RNAs. The number and the exact location of RNA modifications, as well as their stoichiometries for mRNA and other scarce RNA species, are still under debate. Deep sequencing methods are undoubtedly the best suited for extensive mRNA analysis, but their application is not always straightforward, and every method has its own limitations and drawbacks. Newly appearing protocols involving nanopore sequencing are promising, but their application is still very far from routine RNA modification analysis. Most popular second-generation deep sequencing protocols and their application to whole transcriptome are discussed as well as possible general guidelines for protocol validation and application.

The SARS-CoV-2 nucleocapsid protein inhibits the cellular Nonsense-Mediated mRNA Decay (NMD) pathway preventing the full enzymatic activation of UPF1

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The Nonsense-mediated mRNA decay (NMD) pathway triggers the degradation of defective mRNAs and governs the expression of mRNAs with specific characteristics. Current understanding indicates that NMD is often significantly suppressed during viral infections to protect the viral genome. In numerous viruses, this inhibition is achieved through direct or indirect interference with the RNA helicase UPF1, thereby promoting viral replication and enhancing pathogenesis. In this study, we employed biochemical, biophysical assays, and cellular investigations to explore the interplay between UPF1 and the Nucleocapsid (Np) protein of SARS-CoV-2. We evaluated their direct interaction and its impact on inhibiting cellular NMD. Furthermore, we characterized how this interaction affects UPF1's enzymatic function. Our findings demonstrate that Np inhibits the unwinding activity of UPF1 by physically obstructing its access to structured nucleic acid substrates. Additionally, we showed that Np binds directly to UPF2, disrupting the formation of the UPF1/UPF2 complex essential for NMD progression. Intriguingly, our research also uncovered a surprising pro-viral role of UPF1 and an antiviral function of UPF2. These results unveil a novel, multi-faceted mechanism by which SARS-CoV-2 evades the host's defenses and manipulates cellular components. This underscores the potential therapeutic strategy of targeting Np-UPF1/UPF2 interactions to treat COVID-19.

The 3'-5' exoribonuclease ISG20L2 contributes to 3' terminus maturation of 18S and 28S ribosomal RNAs

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Ribosome biogenesis is a nodal process in cell growth and proliferation, and its dysfunction is associated to human congenital diseases and tumorigenesis. Despite extensive mechanistic characterization, several processing steps of human pre-ribosomal RNAs remain elusive. The 47S primary transcript contains 3 out of 4 ribosomal RNAs flanked by external (5'ETS, 3'ETS) and internal (ITS1, ITS2) transcribed spacers. The molecular processes leading to the removal of the 3'ETS, one of the earliest maturation steps, is not fully understood. Combining loss-of-function experiments and 3'-RACE high-throughput sequencing, we showed that the vertebrate-specific 3'-5' exoribonuclease ISG20L2, a DEDDh RNase T superfamily member, is critical for efficient removal of the 3'ETS and formation of large ribosomal subunits. ISG20L2 inactivation led to accumulation of various forms of 3'-extented pre-rRNAs and disorganized PDFC, a sub-nucleolar compartment recently linked to 3'ETS processing. The function of ISG20L2 also extends to the trimming of ITS1 after endonucleolytic cleavage at site 2 and its knockdown reveals the precise location of site 2. Altogether, the present work uncovers the landscape of two pre-rRNA processing steps at nucleotide resolution, and restricts the function of ISG20L2 to the nucleoli where it contributes to the maturation of 18S and 28S 3' ends.

Contribution of Ski Factors to Nascent Peptide Degradation during Ribosome Quality Control

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Aberrant or damaged mRNAs can stall ribosomes during translation and adversely affect cellular functions. Eukaryotic cells exhibit multiple quality control mechanisms to manage problematic protein synthesis. For instance, Ribosome Quality Control (RQC) monitors incomplete nascent peptides associated with such stalled ribosomes. Any failure to complete RQC can result in the production of incomplete and dysfunctional proteins, as well as the formation of potentially toxic cellular aggregates.

In these defective mRNAs, collisions of ribosomes are considered the primary trigger for quality control mechanisms, leading to the degradation of nascent peptides (NPs) along with mRNA decay. Although significant progress has been made in understanding the mechanisms leading to NP degradation, the coordination between NP decay and RNA decay pathways remains unclear. It is proposed that NP degradation coincides with mRNA degradation, offering the advantage of curtailing translation cycles of aberrant messengers, thereby preventing excessive burden on the NP quality control system.

Upon ribosome collisions, problematic mRNAs are primarily degraded through 5'-decapping and 5'-3' exonucleolytic processing by the canonical degradation machinery, with the 5'-3' exoribonuclease Xrn1 playing a major role. When ribosomal collisions are too robust, or in the absence of RQC, Cue2-mediated endonucleolytic cleavage activity may serve as a fail-safe system. Cue2 cleavage, proximal to the stalling site, results in the production of 5_ NGD mRNA intermediates, which are further degraded by the 3'-5' exonucleolytic pathway involving the Ski complex (Ski2, Ski3 & Ski8), Ski7 and the cytoplasmic exosome and in the production of 3_ NGD_mRNA intermediates degraded by the 5'-3' exoribonuclease Xrn1.

Comprendre via proximity labeling la regulation par la chromatine de l'epissage dependant de PTBP1

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L'epissage alternatif est un processus essentiel pour la diversite proteique et l'identite cellulaire, dont une deregulation peut mener au cancer. Recemment nous avons decouvert un role des marques d'histone permettant de reguler de maniere dynamique l'epissage lors de la transition epithelio-mesenchymateuse (EMT) [1]. Ainsi il est montre que la marque H3K27ac permet le recrutement d'un facteur d'epissage essentiel, PTBP1, alterant ainsi l'epissage de ce locus specifique. Des etudes ChIP-seq et RNA-seq sur le genome entier ont montre que parmi les evenements d'epissage PTB-dependant et changeant au cours de l'EMT, 13% presentent egalement des changements de niveau de H3K27ac, contre seulement 4% de H3K4me3. Pour verifier cette proximite, j'ai realise des IF de haute resolution et des PLA entre PTBP1 et H3K27ac, ou entre PTBP1 et H3K4me3. Enfin, pour trouver le lien mecanistique entre H3K27ac et PTBP1, j'utilise une technique innovante de proximity labeling basee sur l'utilisation d'un anticorps. Apres ciblage d'une biotine ligase APEX a PTBP1 via cet anticorps, je serai en mesure d'identifier toutes les proteines a proximite. En couplant cela a un fractionnement cellulaire pour isoler la bulle chromatinienne, ainsi qu'a un proximity labeling sur H3K27ac, et en les croisant avec des donnees pangenomiques, je serai en mesure de comprendre le role de cette marque d'histone et de ses proteines regulatrices dans l'epissage dependant de PTBP1 au cours de l'EMT. [1] Segelle et al., Cell Rep 2022

RNA dysregulation and disrupted cell identity in senataxin-associated motor neuron disease

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Transcription is crucial for gene expression but can interfere with DNA replication and repair if not terminated properly. Transcription is also a source of R-loops, structures formed when the nascent RNA invades and anneals abnormally with the template DNA. Persistent R-loops can cause DNA damage and genome instability, and thus, various factors control R-loop levels to limit their pathological roles. In humans, RNA/DNA helicase senataxin (SETX) has been attributed an important role in transcription termination and R-loop resolution. Mutations in the SETX gene cause a juvenile form of Amyotrophic Lateral Sclerosis named ALS4 that is characterized by motor neuron (MN) degeneration. However, the molecular pathways linking SETX mutation to MN impairment remain mysterious. To fill this gap, we have characterized the impact of a penetrant ALS4 mutation in human MNs generated by differentiation of induced pluripotent stem cells. Importantly, we found that this mutation induces a reduction of axon length over time, as observed in other ALS models. Consistent with the observed axonal impairment, ALS4 MNs exhibited substantial downregulation of numerous genes involved in axon function. In addition, we detected overexpression and overactivation of the TGF-i_ signaling pathway in ALS4 MNs, mirroring observations in ALS4 patients and other ALS forms. In agreement with this aberrant activation, we also observed upregulation of numerous genes induced by the TGF-i_ pathway that are not normally expressed in MNs such as genes involved in the development and function of the cardiovascular and skeletal systems. In addition, genes promoting cell cycle progression appeared activated in mutant MNs, suggesting compromised post-mitotic fate, a feature that we found to be shared by other ALS types. Moreover, we detected hundreds of mRNAs exhibiting substantial splicing alterations without changes in their expression levels, including many that are important for axon integrity and function. Finally, we did not observe typical consequences of SETX loss of function such as the accumulation of R-loops and DNA damage, supporting the idea that ALS4 mutations induce a toxic gain of function. We propose that the disruption of MN identity, driven by dysregulated RNA expression and processing, is a key pathogenic event in SETX-associated ALS and likely other forms of the disease. Our findings shed new light on the molecular pathways involved in ALS and highlight potential targets for future therapeutic strategies.

Transcriptional regulation by the 7SK snRNP during neuronal differentiation

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On most genes, RNA polymerase II is deliberately paused after the transcription start site by two negative elongation factors, DSIF and NELF. The transition to productive elongation relies on the positive transcription elongation factor b (P-TEFb), which is composed of the CDK9 kinase and Cyclin T1. In cells, the level of CDK9 activity dictates the fraction of RNAPII that overcomes pausing and synthesizes full-length mRNA [1,2]. The activity of P-TEFb is primarily controlled by the 7SK snRNA that sequesters P-TEFb into a kinase-inactive 7SK/P-TEFb RNP. The 7SK snRNP controls the availability of P-TEFb through releasing or capturing active P-TEFb according to the transcriptional requirements of the cell [3,4]. During differentiation, cells acquire specialized transcriptional programs in order to be directed into appropriate lineages. RNAPII pausing is essential to this process, as disruptions in pausing have been shown to affect crucial stages of cell-identity specification in Drosophila and Mouse [5,6]. How P-TEFb activity and RNAPII pausing are modulated during cell differentiation is not well understood, but 7SK snRNA expression is higher in differentiated cells, especially in the central nervous system. In addition, loss of the 7SK chaperone LARP7, which reduces 7SK snRNA levels, leads to Alazami syndrome, a neurodevelopmental disorder characterized by intellectual disability, short stature, and distisntive facial features [7]. These data suggest that the 7SK snRNP may be critical for brain development. My PhD project aims at exploring the fusntion of 7SK snRNA in controlling P-TEFb activity, RNAPII pausing and gene expression during neuronal differentiation. The human embryonic pluripotent carcinoma stem cell line NT2/D1 (NT2), when exposed to retinoic acid (RA), can differentiate into neurons, making them a convenient in vitro neuronal differentiation model. [8]. We generated NT2 knockout cell lines with CRISPR-Cas9 to better understand the impact of the 7SK snRNP on RNAPII transcription during differentiation, using transcriptomic approaches (ChIP-seq, RNA-seq). In parallel, the requirement of the 7SK snRNP for neocortex formation is being assessed using 3D brain organoids derived from human induced pluripotent stem cells (iPSCs). [1]. Egloff S (2021). CDK9 keeps RNA polymerase II on track. Cell Mol Life Sci 78, 5543–5567. [2]. Leighton Core and Karen Adelman. Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. Genes Dev. 2019 Aug 1; 33(15-16): 960 982 [3]. V. T. Nguyen, T. Kiss, A. A. Michels, O. Bensaude, 7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complexes. Nature 414, 322-325 (2001). [4] S. Egloff, E. Van Herreweghe, T. Kiss, Regulation of polymerase II transcription by 7SK snRNA: two distisnt RNA elements direct P-TEFb and HEXIM1 binding. Molecular and cellular biology 26, 630-642 (2006). [5] Mounia Lagha, Jacques P Bothma and all. Paused Pol II coordinates tissue morphogenesis in the Drosophila embryo. Cell 2013 May 23 ;153(5): 976-87. [6] Amleh A, Nair SJ, Sun J, Sutherland A, Hasty P, et al. (2009) Mouse Cofactor of BRCA1 (Cobra1) Is Required for Early Embryogenesis. PLoS ONE 4(4): e5034.
[7]. A. M. Alazami and al., Loss of fusntion mutation in LARP7, chaperone of 7SK snRNA, causes a syndrome of facial dysmorphism, intellectual disability, and primordial dwarfism. Hum Mutat 33, 1429-1434 (2012) [8] S. Abolpour Mofrad, K. Kuenzel, O. Friedrich, D. F. Gilbert, Optimizing neuronal differentiation of human pluripotent NT2 stem cells in monolayer cultures. Dev Growth Differ 58, 664-676 (2016).

Pathogenic minor intron retentions in rare genetic diseases: what long-read sequencing can tell us

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Alternative splicing is a key process that allows multiple mRNA isoforms to be generated from a single gene, ensuring a diversity of transcripts expressed in different tissues or developmental stages. Intron retention (IR) is a class of alternative splicing event that was initially less studied than the other events due to the difficulty of measuring them reliably. However, a growing number of studies are evidencing the role of IR in the regulation of various cellular processes, as well as mechanisms that result in aberrant IR in certain pathologies. In most eukaryotes, introns can be classified into two groups based on their distinct consensus splice site and branch point sequences. The major introns represent the vast majority of introns, while the minor introns account for less than 1% of them. The major and minor spliceosomes are the two ribonucleoproteic complexes that ensure their respective recognition and excision. Human embryonic development relies on minor intron splicing. Indeed, biallelic mutations in RNU4ATAC, a non-coding gene transcribed into the minor spliceosome component snRNA U4atac, are responsible for a group of rare microcephalic osteodysplastic growth restriction syndromes. The team used short-reads bulk RNA sequencing to conduct previous transcriptomic analysis on multiple models, including TALS patients derived cells, neural progenitors and neurons that were differentiated from iPS cells with two patient recurrent mutations in U4atac, and zebrafish morphant models. As expected, minor intron retention was strong in each of these models. Despite dedicated bioinformatics tools, short read bulk RNA sequencing is limited in its ability to reliably quantify IR due to various reasons; in particular the fact that numerous genes are transcribed in multiple overlapping transcripts which may distort the quantification of introns overlaid by exons. By sequencing individual transcript molecule in full, long read RNA sequencing enables precise identification of IR. Thus, in order to overcome limitations of short read RNA-seq and to further understand the mechanisms controlling the splicing of minor introns in the presence of RNU4ATAC mutations, we performed long read sequencing of RNA extracted from cells from three TALS patients and five matched controls. We will discuss the comparison of this analysis with short-read bulk RNA sequencing carried out on the same samples.

Study of the link between RNA degradation and energy metabolism in Escherichia coli

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Background: Bacteria require ATP for cell survival. ATP is obtained from the catabolism of carbon substrates to provide energy for growth and cell maintenance[1]. Preliminary modelling results obtained in collaboration with INRIA have shown that the proportion of energy used for cell maintenance is greater for non-preferential carbon sources than for preferential carbon sources. Fructose and xylose are non-preferential carbon sources[2] used in biotechnologies and on which E. coli growth and productivity can be limited. To overcome these limitations, our goal is to reallocate energy intended for a cell maintenance process, such as the RNA life cycle, to growth and productivity. In this study, the role of three RNA degradation enzymes – the endoribonuclease E and the 3'-exoribonucleases II and R – was investigated on several physiological parameters of E. coli during growth on preferential and non-preferential carbon sources. Methods: In this work, the growth rate, cell density at the beginning of the stationary phase, intracellular ATP concentration (by enzymatic assay) and glycogen concentration (by iodine vapor staining and enzymatic assay) were characterized in the parental E. coli strain BL21(DE3) and in mutants with single or combined deletions of the rnb and rnr genes coding for RNase II and RNase R, respectively, and a truncation of the rne gene leading to the deletion of the C-terminal domain of RNase E and the disruption of the degradosome. The physiological characterization was carried out on minimal medium (M9) with fructose, xylose or glucose as the sole carbon source. Results: Disruption of RNases II, E and R had significant effects on E. coli physiology. The single or combined mutants with rne1_598 mutation are particularly interesting with an increase in growth rates on xylose, but a slow down on fructose. In addition, these mutants accumulate glycogen on glucose and their intracellular ATP concentration is higher on fructose. On xylose, only the single rne1-598 mutant and the double _rnb/rne1-598 mutant show an increase in ATP concentration. Finally, the _rnb, _rnr and _rnb/_rnr mutants enter stationary phase at a higher cell concentration on both fructose and xylose. A hierarchical clustering of these phenotypic data was performed to formally examine physiological similarities and differences between the strains. Conclusion: These results contribute to the characterization of the link between RNA degradation and energy metabolism in Escherichia coli. They highlight the involvement of RNase E in glycogen storage and intracellular ATP concentration regulation, as well as the role of RNases II and R in cell concentration determination in stationary phase. To better understand these phenotypes, omics analyses, including transcriptomics to determine RNA concentrations and half-lives, and metabolomics to quantify all nucleotide pools, need to be performed. [1]Pirt, J. The maintenance energy of bacteria in growing cultures. Proc. R. Soc. Lond. B. 163, 224–231 (1965). [2]Wang, X., Xia, K., Yang, X. & Tang, C. Growth strategy of microbes on mixed carbon sources. Nat. Commun. 10, 1279 (2019).

Investigation of miR-277 and Ago in Insects: Analysis of Similarities and 3' Methylation Conservation of microRNAs

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MicroRNAs (miRNAs) are key post-transcriptional regulators in many biological processes of various organisms, including insects. Among these miRNAs, miR-277 plays an important role in regulating energy metabolism, particularly in Drosophila melanogaster (1). This work is part of a comparative approach aimed at exploring the similarities between the sequences of this miRNA and the Ago2 protein of D. melanogaster and other insect species, while investigating the conservation of 3' methylation of miRNAs loaded onto Argonaute 2 (Ago2), whose potential biological functionality that is still poorly understood (2). The bioinformatics analysis begins with the search for orthologs of miR-277 and Ago proteins across several insect clades using available genomic data from NCBI. The conservation of miR-277 was determined, and the search for Ago proteins, particularly those similar to D. melanogaster's Ago2, was performed using a HMMer approach on the predicted proteomes of various species (3). These analyses identified insect species that potentially carry functionally conserved miR-277 and Ago2. Once the orthologs were identified, an experimental approach is considered to evaluate the 2'-O-methylation of the 3' end of miR-277 in different insect species using a molecular biology technique: sodium periodate oxidation prior to sequencing, allowing for the distinction between small RNAs with or without a modified 3' end (4). From the obtained oxidized and non-oxidized Small RNA-Seq libraries, data will allow the study of miRNA 3' methylation within a broader phylogenetic framework. This bioinformatics approach will serve as a springboard for future experiments aimed at confirming the methylation of identified miRNAs, thereby facilitating the understanding of evolutionary differences in miRNA regulation between species. References: [1] Forstemann K, Horwich MD, Wee L, Tomari Y, Zamore PD. Drosophila microRNAs are sorted into functionally distinct argonaute complexes after production by dicer-1. Cell. 2007 Jul 27;130(2):287-97. doi: 10.1016/j.cell.2007.05.056. PMID: 17662943; PMCID: PMC2686109. [2] Horwich, M. D., Li, C., Matranga, C., Vagin, V., Farley, G., Wang, P., et Zamore, P. D. (2007). The Drosophila RNA methyltransferase, DmHen1, modifies germline piRNAs and single-stranded siRNAs in RISC. Curr Biol, 17(14) : 1265–1272. [3] Pinzón N, Bertrand S, Subirana L, Busseau I, Escrivá H, Seitz H. Functional lability of RNA-dependent RNA polymerases in animals. PLoS Genet. 2019 Feb 19;15(2):e1007915. doi: 10.1371/journal.pgen.1007915. PMID: 30779744; PMCID: PMC6396948. [4] Ghildiyal M, Seitz H, Horwich MD, Li C, Du T, Lee S, Xu J, Kittler EL, Zapp ML, Weng Z, Zamore PD. Endogenous siRNAs derived from transposons and mRNAs in Drosophila somatic cells. Science. 2008 May 23;320(5879):1077-81. doi: 10.1126/science.1157396. Epub 2008 Apr 10. PMID: 18403677; PMCID: PMC2953241

Extensive uORF translation from HIV-1 transcripts elicits specific T cell immune responses in infected individuals and conditions DDX3 dependency for expression of main ORFs

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Human immunodeficiency virus type-1 (HIV-1) is a complex retrovirus which relies on alternative splicing, translational and post-translational mechanisms to produce more than 15 functional proteins from its single ~10kb transcriptional unit. Here, we have applied, RNA-seq, ribosome profiling, nascent protein labelling and quantitative mass spectrometry at different time points during infection of CD4+ T lymphocytes to characterize the translational landscape of cellular and viral transcripts during the course of infection. Our results indicate a strong impact of viral infection on host cellular transcript levels but a modest impact on global translation rates. Interestingly, analysis of ribosome profiling reads from viral transcripts reveals extensive and productive non-AUG translation of small peptides from multiple upstream open reading-frames (uORFs) located in the 5' long terminal repeat. Remarkably, uORF-derived peptides elicit specific T cell responses in people living with HIV suggesting a potential role in the progression of the disease. uORF translation is conserved among other retroviruses and, together with the TAR sequence, condition the dependency on DDX3 for efficient translation of the main viral open-reading frames.

MAPK pathway-regulated function of RBM34 in the nucleolar stages of the synthesis of the human 60S ribosomal subunit

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Ribosome biogenesis involves the synthesis by RNA polymerases I and III of ribosomal RNA (rRNA) precursors, their packaging into precursor particles (pre-ribosomes), their modification, processing and association with ribosomal proteins (RPs) to generate the mature ribosomal subunits. This process requires scores of assembly factors (AFs) that transiently interact with pre-ribosomes to fulfill specific functions in the maturation process. Signal transduction cascades tightly regulate this process to adapt ribosome biogenesis to cell growth requirements. Alterations in these signalling pathways promote unrestrained ribosome production and increased global protein synthesis that are critical features of cancer initiation and progression. The MAPK pathway regulates ribosome production at different stages, including transcription by RNA polymerases I and III and translation of ribosomal proteins (RPs). Our recent data suggest that the MAPK pathway also regulates the co- and post-transcriptional stages of ribosome synthesis, to coordinate the synthesis of the primary transcripts, their packaging into pre-ribosomes and the whole maturation process. In this work, we are addressing the role of RSK kinases, the downstream effectors of the MAPK signalling pathway, in the maturation of pre-60S particles, the precursors to the large ribosomal subunits. We have identified RBM34, a protein involved in the early, nucleolar stages of this process, as a RSK substrate and showed that some phosphorylation events of RBM34 are under the control of the MAPK pathway. We are now investigating the function of RBM34 in the maturation of pre-60S particles and the impact of its regulation by the MAPK pathway. This work will lead to a comprehensive view of the impact of the MAPK signaling pathways on the co- and post-transcriptional stages of ribosome biogenesis in human cells.

Identification of CK1δ/ε as the functional equivalent of SMG1 in yeast nonsense-mediated mRNA decay

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Nonsense-mediated mRNA decay (NMD) is a highly conserved mechanism that removes unwanted transcripts from eukaryotic cells, including the products of pervasive transcription, developmentally regulated mRNAs or viral RNA. Despite the high conservation of core NMD factors, SMG1, a protein kinase that phosphorylates UPF1 and is essential for NMD in mammals, has no known equivalent in S. cerevisiae. We present evidence that the ribosome biogenesis factor Hrr25, the yeast equivalent of the mammalian protein kinase CK1δ/ε, is the functional equivalent of SMG1. Hrr25 is associated with complexes involved in cytoplasmic NMD, in particular those containing the decapping complex and the core NMD factor Upf1. The association of Hrr25 with Upf1 led us to investigate the phosphorylation of this central NMD factor, whose reversible modification has been linked to its function in NMD in other organisms. We identified a phosphorylation site in the C-terminal region of Upf1 and showed that its modification depends on Hrr25. The changes in Upf1 phosphorylation were similar to those observed for Upf1 in other organisms, decreasing in the absence of Upf2 and increasing in the absence of Ebs1 (SMG5/7 equivalent). Inhibition of Hrr25 kinase activity resulted in the stabilisation of an NMD reporter mRNA and an increase in the levels of endogenous transcripts targeted by NMD. We also found that Hrr25 inhibition resulted in a strong defect in nuclear RNA degradation, affecting transcripts generated by all three RNA polymerases. We propose that Hrr25 is the long-sought functional analogue of the metazoan protein kinase SMG1 for yeast NMD. Our results further support a key role for the CK1δ/ε family of protein kinases in coordinating RNA quality control at multiple levels in eukaryotes.

Ultraconserved mechanisms of ribosome isoaspartylation

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All living beings use ribosomes to synthesise proteins. While the ribosome structure and components are overall conserved, ribosome assembly pathways have diverged considerably between bacteria, archaea, and eukaryotes. Indeed, there remain only few universally conserved biogenesis steps, primarily serving to install some invariant ribosomal RNA modifications required for translation in most species. Recent studies suggested that ribosomal proteins may also be subject to broadly conserved modifications likely inherited from the earliest forms of life. For example, the universal ribosomal protein uS11 has been shown to undergo site-specific isoaspartylation in various organisms [1-5]. However, the molecular mechanism and the biological significance of this phenomenon remain obscure. Here, using structural, biochemical, and genetic approaches, we show that two independently evolved highly conserved enzymes catalyse the installation of an isoaspartate residue in the C-terminal tail of uS11 in most existing ribosomes. In bacteria and their derivatives, mitochondria and plastids, the putative ribonuclease YbeY converts an invariant asparagine into isoaspartate through a mechanism partially emulated on that of zinc-dependent proteinases. Similarly, in archaea and their descendants, eukaryotes, the atypical kinase Fap7 isomerises an aspartate in the same position of uS11 using a totally different, ATP-dependent, activation mechanism. We show that, in either case, the isoaspartylation is functionally critical: this strategically positioned modification radically changes the geometry of the peptide chain of uS11 and enables its correct incorporation into the platform of the ribosomal small subunit, which is required for efficient translation. Inability of a cell to isoaspartylate uS11 leads to ribosome destabilisation, a dramatic impairment of protein synthesis, pleiotropic metabolic perturbations, and a severe growth retardation, in many cases culminating in lethality. Therefore, uS11 isoaspartylation represents one of the few extant universally conserved and functionally essential ribosome biogenesis mechanisms. [1] Watson ZL, Ward FR, Meheust R, Ad O, Schepartz A, Banfield JF, Cate JHD (2020) Structure of the bacterial ribosome at 2 Å resolution. eLife 9:e60482. [2] Itoh Y, Singh V, Khawaja A et al. (2022) Structure of the mitoribosomal small subunit with streptomycin reveals Fe-S clusters and physiological molecules. eLife 11:e77460 [3] Cottilli P, Itoh Y, Nobe Y et al. (2022) Cryo-EM structure and rRNA modification sites of a plant ribosome. Plant Commun 3:100342. [4] Pellegrino S, Dent KC, Spikes T, Warren AJ (2023) Cryo-EM reconstruction of the human 40S ribosomal subunit at 2.15 Å resolution. Nucleic Acids Res, 51, 4043–4054. [5] Holvec S, Barchet C, Lechner A et al. (2024) The structure of the human 80S ribosome at 1.9_Å resolution reveals the molecular role of chemical modifications and ions in RNA. Nat Struct Mol Biol 31, 1251–1264.

snoFlake: Discovery of a snoRNA-guided Splicing Regulatory Complex via the snoRNA-RBP Interactome

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Box C/D small nucleolar RNAs (snoRNAs) are noncoding RNAs crucial for guiding 2'-O-ribose methylation in ribosomal RNA during ribosome biogenesis, primarily through the formation of ribonucleoprotein (snoRNP) complexes with core RNA-binding proteins (RBPs) [1,2]. Additional roles were proposed for box C/D snoRNAs, including the regulation of alternative splicing of protein-coding transcripts, yet few validated examples exist, with unclear mechanisms. Some noncanonical functions are thought to involve interactions with additional RBPs beyond the core snoRNA binders, indicating diverse regulatory roles of snoRNAs by interacting with various RBPs, collectively modulating protein-coding target RNAs. To explore these interactions and their functional implications, we introduce snoFlake (snoRNA-RBP Functional Interaction Network Models), an interaction network of 208 box C/D snoRNAs and 166 human RBPs, showing direct binding interactions and significant overlap of binding sites (p-value $< 1.64e-04$) on shared protein-coding target RNAs, reinforcing their concerted role in gene regulation. Focusing on snoRNAs targeting groups of functionally-related targets, also bound by snoRNA-associated RBPs led to a hub region composed of SNORD22 and U5-associated splicing factors: PRPF8 and EFTUD2. SNORD22, PRPF8 and EFTUD2 exhibited an enrichment of overlapping binding sites at both 5' and 3' splice sites, suggesting their involvement in a splicing regulatory model. Knockdown experiments and differential alternative splicing analysis further highlighted the potential role of the SNORD22-PRPF8-EFTUD2 complex in splicing, marking the first snoRNP splicing regulatory complex. This reshapes the understanding of snoRNA biology, emphasizing snoFlake's potential as a foundation for unravelling the impact of snoRNA-RBP interactions in gene regulation. [1] Bergeron et al. Biochemical Society Transactions (2020). [2] Bratkovic et al. Nucleic Acids Research (2020).

NONO and INO80 are new clients of the HSP90/R2TP system and their assembly into complex depends on RPAP3 subunit

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The HSP90 chaperone, together with its co-chaperone R2TP, assists the assembly of various multi-subunit machineries, including the three RNA polymerases and several non-coding RNPs, such as snoRNPs, snRNPs and miRNPs. The R2TP complex is composed of RUVBL1/2, two AAA+ ATPases that possess their own chaperone activity and two adaptor subunits, RPAP3 and PIH1D1. PIH1D1 has been shown to directly recruit clients of the R2TP chaperone. In contrast, whether RPAP3 can also specifically recruit R2TP clients is still unclear. To systematically characterize the interactome of RPAP3, we performed a two-hybrid screen and a MS-based proteomic analysis of RPAP3 interaction partners after RPAP3 immunoprecipitation (IP). Many newly identified interactions were then validated by LUMIER IP. To decipher the functional importance of these interactions, we analyzed the expression level of the newly identified putative R2TP clients in RPAP3-depleted cells, e.g., RPAP3-AID cells generated by CRISPR/Cas9, using a luciferase assay, and identified several new R2TP clients, whose expression level is altered by RPAP3 depletion. An inhibitor of HSP90, geldanamycin, also affects the level of numerous tested clients, showing that HSP90 and RPAP3 work in concert to favor the stability and the assembly of their clients in multiprotein complexes. We then focused our study on two of the newly identified RPAP3 partners, NONO and INO80, which are a paraspeckle component and a member of a chromatin remodeling complex, respectively, to understand how R2TP is involved in their assembly into complexes. By Affinity Purification coupled to Mass Spectrometry (AP-MS), we showed that RPAP3 depletion leads to a decreased association of NONO and INO80 with their known partners, underlying the role of RPAP3 in formation of these complexes. AP-MS of RUVBL1 in RPAP3-depleted cells showed that some R2TP clients are more sensitive than others to the absence of RPAP3. Altogether, these results show that the HSP90/R2TP chaperone has a very large number of clients that have not been described so far and that their assembly in complexes can depend on RPAP3 subunit for their recruitment by R2TP, independently of their binding to HSP90.

Regulation of the Activity of TAR-RNA Binding Protein (TRBP) by RNA Polymerase II Associated Protein 3 (RPAP3)

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TRBP is a protein involved in various biological pathways, such as miRNA maturation or HIV replication.
Indeed, TRBP is one of Dicer's possible co-factor, and it ensures efficient Dicer processing of the pre-miRNA.On the other hand, TRBP induces HIV replication by relieving the inhibitory effect of the TAR RNA element on the translation of downstream viral genes.
We recently showed that the R2TP chaperon complex component RPAP3 directly binds TRBP and we obtained the crystal structure of this complex, identifying by the way key residues involved in the interaction. Remarkably, binding of TRBP to RPAP3 or Dicer is mutually exclusive.
In this project, we will study the possible functions RPAP3 might play in the regulation of TRBP activity, notably using RPAP3 depleted cells and expression of RPAP3 mutants. We already showed that TRBP activity is improved both for miRNA's processing and TAR-dependent translation when RPAP3 is degraded, suggesting its regulatory role for TRBP activities. However, further complementary experiments will be performed to better understand the mode of action of RPAP3.

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