9ème colloque

# RNA Occ'

Club RNA Occitanie

## 17 Juin 2025

**Amphitheatre, CNRS DR13**
**1919 route de Mende**
**34396 Montpellier**

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| 09h15 - 9h55 | **Welcome Participants** |
| 10h00 - 11h00 | **Invited Speaker**<br>*Chair: Isabel Chillon, IGMM, Montpellier*<br><br>**Jorge FERRER** (CRG, Barcelona)<br><br>***An RNA-Centric View of the Metabolic Gatekeeper HNF1A*** |
| 11h00 - 11h30 | **Coffee break** |
| | **SESSION 1 – Transcriptional and Post-Transcriptional Processes in the Nucleus**<br>*Chairs: Charlotte Grimaud, IGH, Montpellier & Julie Carnesecchi, IGMM, Montpellier* |
| 11h30 - 11h45 | **Flavia Mazzarda** (IGH, Montpellier)<br>*Deciphering the inner workings of the HIV-1 promoter using MS2 imaging and SMF* |
| 11h45 - 12h00 | **Nazim Sarica** (IGH, Montpellier)<br>*A multiplexed quantitative ddPCR approach to study hepatitis B virus transcriptional and post transcriptional regulation* |
| 12h00 - 12h15 | **Ruoyang Zhai, BGI, France**<br>*Enter the Multi-Omics Era with BGI Genomics: Technical Highlights* |
| 12h15 - 12h30 | **Wan Xiang** (IGMM, Montpellier)<br>*The RNA binding activity of the Drosophila Hox Transcription Factor Ultrabithorax contributes to muscle morphogenesis in developing embryos* |
| 12h30 - 12h45 | **Margot Lugoboni** (iGReD, Clermont-Ferrand)<br>*Transcriptional regulation mechanisms mediated by $m^6A$ RNA modification in Drosophila melanogaster* |
| 12h45 – 14h30 | **LUNCH & POSTER SESSION** |
| | **SESSION 2 – From RNA Biogenesis to Translation**<br>*Chairs: David Lleres, CBS, Montpellier & Anne-Catherine Prats, I2MC, Toulouse* |
| 14h30 - 14h45 | **Chloé Bessiere** (CRCT, Toulouse)<br>*Long-read sequencing identifies full-length circular RNAs linked with therapy resistance in pediatric cancers* |
| 14h45 - 15h00 | **Julia Schaeffer** (UGA, Grenoble)<br>*An issue of translation: gene regulation mechanisms to unlock neuron regeneration* |
| 15h00 - 15h15 | **Javier Florido** (IRCM, Montpellier)<br>*Understanding the epitranscriptomic landscape regulated by the p53 pathway* |
| 15h15 - 15h30 | **Léo Le Tareau** (CRBM, Montpellier)<br>*A lncRNA-RNase mechanism at the center of the elimination process of nucleolus-related inclusions* |
| 15h30 - 16h00 | **Coffee Break** |
| | **SESSION 3 – RNA Biology and Infection**<br>*Chairs: Flavia Mazzarda, IGH, Montpellier & Yves Henry, CBI, Toulouse* |
| 16h00 - 16h15 | **Damien Avinens** (IRIM, Montpellier)<br>*Deciphering the translation dynamics of SARS-CoV-2 UTRs* |
| 16h15 - 16h30 | **Beata Jady** (MCD, Toulouse)<br>*Epstein-Barr virus v-snoRNA1 modulates translation of the infected host cell* |
| 16h30 -16h45 | **Kuldeep Lahry** (IRCM, Montpellier)<br>*Microbial metabolite preQ1 regulates host cell physiology through selective tRNA degradation and translation regulation* |
| 16h45 -17h00 | **Johannes Keisers** (CBS, Montpellier)<br>*mRNA degradation as a rescue mechanism for translation-inhibiting antibiotics* |
| 17h05 -17h15 | **Presentation of Awards and Conclusion** |

# Invited Presentation

## *An RNA-Centric View of the Metabolic Gatekeeper HNF1A*

**Jorge Ferrer**

*Regulatory Genomics and Diabetes Lab, Centre for Genomic Regulation, Barcelona*
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Diabetes mellitus afflicts more than 400 million people. Current strategies to prevent and treat diabetes are limited by our scant knowledge of the molecular defects that cause diabetes. We focus on understanding changes in genome regulation that lead to monogenic and polygenic diabetes. We study the gene networks that are essential for insulin-producing beta cells to maintain glucose homeostasis, and develop strategies to manipulate these networks in human patients. We are also interested in how gene regulatory mechanisms can be harnessed for regenerative therapies in autoimmune diabetes. To achieve these goals, we combine regulatory genomics, human genetics, and genome engineering in model systems.

https://www.crg.eu/es/programmes-groups/ferrer-lab

Oral presentation

# Deciphering the inner working of HIV-1 promoter using MS2 imaging and SMF

**Vera Saninova[1*], Flavia Mazzarda[1*], Lasha Dalakishvili[2], David Depierre[3], Christina J. I. Moene[4], Rachel Topno[1,5], Jean-Marc Escudier[3], Marie-Cécile Robert[1], Olivier Cuvier[3], Arnaud Krebs[4], Nacho Molina[2], Ovidiu Radulescu[5], Edouard Bertrand[1]**

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Despite significant advances in HIV-1 research, a cure remains elusive due to the persistence of a latent viral reservoir. This reservoir forms early in infection and is sustained by low-level viral replication, even under effective antiretroviral therapy (ART)[1]. These cells evade both ART and the immune system detection, making viral persistence lifelong[2]. Discontinuation of ART leads to viral rebound within weeks, necessitating lifelong treatment with risks of side effects and possible arise of resistant strains2. Consequently, targeting the latent reservoir is critical for achieving a cure. Two main therapeutic strategies are under development to target HIV-1 latency: "block and lock" and "shock and kill." The first approach aims to suppress residual HIV-1 transcription, locking the virus into a deep latent state. In contrast, "shock and kill" utilizes latency-reversing agents (LRAs) to reactivate viral transcription, offering promise but facing significant challenges in fully eradicating the reservoir[3]. One of the main obstacles to this approach is the inherent stochasticity of transcription. Live-cell RNA imaging has revealed that transcription levels are encoded by the intrinsic dynamics of promoters, which stochastically switch between transcriptionally active and inactive states[4-6]. In 2013, it was demonstrated in patient derived cells that latency in HIV-1 is influenced by this stochasticity, with only a subset of latent cells becoming activated after LRA treatment, and repeated treatments reactivating new latent viruses[7]. Understanding the factors that control viral transcription in latent cells is crucial for developing more effective antiviral strategies. A central regulator of HIV-1 transcription is the viral activator Tat. Tat enhances transcription via a positive feedback loop and can amplify viral gene expression up to 1000-fold by: i) binding to the nascent TAR RNA transcribed by initiating polymerases[8], ii) recruiting the P-TEFb complex composed of Cyclin T1 and CDK9[9-11], and iii) remodeling the nucleosome structures of the HIV-1 promoter[12-15], collectively promoting elongation. When Tat concentration is high, transcription is fully activated, resulting in acute infection; at low concentrations, transcription is suppressed, leading to latency. Here, we combined Single Molecule DNA footprinting (SMF) and MS2-based live-cell transcription imaging to provide a mechanistic model of promoter dynamics, capturing both kinetic and molecular aspects. SMF shows that the HIV-1 promoter functions in two modes depending on Tat presence. Without Tat, a DHS1 nucleosome blocks the core promoter where the pre-initiation complex (PIC) assembles. In presence of Tat, the DHS1 nucleosome is mostly absent while TBP and initiating polymerases are frequently detected. Modeling together the MS2 and SMF data provides the rates of DHS1 nucleosome deposition and removal, PIC assembly and polymerase loading. The data further reveal a kinetic proofreading mechanism, linking immobilized initiating polymerases to the closure of the HIV-1 promoter. This mechanism prevents spurious transcription and enables Tat to indirectly control promoter nucleosomes by promoting elongation, therebyboosting viral transcription. By modeling together data from SMF and MS2 imaging, we provide, for the first time, a molecular description of the dynamics of a promoter, revealing its mechanisms of action and a key step of kinetic proofreading.

*[1] Lorenzo-Redondo, R. et al. Persistent HIV-1 replication maintains the tissue reservoir during therapy. Nature 520, 51-56 (2016).*
*[2] Pierson, T., McArthur, J. & Siliciano, R. F. Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy. Annu. Rev. Immunol. 18, 665-708 (2000).*
*[3] Lichterfeld, M. Reactivation of latent HIV moves shock-and-kill treatments forward. Nature 578, 42-43 (2020).*
*[4] Tantale, K. et al. A single-molecule view of transcription reveals convoys of RNA polymerases and multi-scale bursting. Nat. Commun. 7, 12248 (2016).*
*[5] Tantale, K., et al. Stochastic pausing at latent HIV-1 promoters generates transcriptional bursting. Nature communications, 12, 4503. (2021).*
*[6] Chubb, J., Trcek, T., Shenoy, S. & Singer, R. Transcriptional pulsing of a developmental gene. Curr Biol. 16, 1018-1025 (2006).*
*[7] Ho, Y. et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell. 155, 540-551, doi:0.1016/j.cell.2013.09.020 (2013).*
*[8] Cary, D. C., Fujinaga, K. & Peterlin, B. M. Molecular mechanisms of HIV latency. J. Clin. Invest. 126, 448-454 (2016).*
*[9] Core, L. J. & Lis, J. Transcription regulation through promoter-proximal pausing of RNA polymerase II. Science 319, 1791-1792(2008).*
*[10] Danko, C. et al. Signaling pathways differentially affect RNA polymerase II initiation, pausing, and elongation rate in cells. Mol Cell. 50, 212-222 (2013).*
*[11] Kwak, H. & Lis, J. Control of transcriptional elongation. Annu Rev Genet. 47, 483-508 (2013).*
*[12] Benkirane, M. et al. Activation of integrated provirus requires histone acetyltransferase. p300 and P/CAF are coactivators for HIV-1 Tat. J Biol Chem 271, 24898-24905 (1998).*
*[13] Marzio, G., Tyagi, M., Gutierrez, L. I. & Giacca, M. HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. Proc Natl Acad Sci U S A. 95, 13519-13524 (1998).*
*[14] De Crignis, E. & Mahmoudi, T. Int Rev Cell Mol Biol. The Multifaceted Contributions of Chromatin to HIV-1 Integration, Transcription, and Latency 328, 197-252 (2017).*
*[15] Marcello, A., Zoppé, M. & Giacca, M. Multiple modes of transcriptional regulation by the HIV-1 Tat transactivator. IUBMB Life 51, 175–181 (2001).*

Oral presentation

# A multiplexed quantitative ddPCR approach to study hepatitis B virus transcriptional and post transcriptional regulation

**Nazim SARICA, Irene MEKI, Gael PETITJEAN, Christine NEUVEUT**

*Molecular Virology Lab, Institut de Génétique Humaine, Montpellier*
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Chronic hepatitis B virus (HBV) infection that affects more than 250 millions of persons remains a human threat responsible for the development of severe liver diseases such as cirrhosis and liver cancer. HBV replication and persistence rely on the transcription of the viral episomal nuclear DNA called cccDNA that is about 3,2kbp. cccDNA is the template for the transcription of all the viral transcripts including the pregenomic RNA (pgRNA) that is encapsidated in the cytoplasm and retrotranscribed into viral DNA. Understanding cccDNA transcriptional regulation and the post- transcriptional regulation of HBV transcripts is thus a key step in the race to develop new curative strategies and achieve HBV cure. cccDNA genome contains 4 overlapping open reading frames coding for 6 major RNAs: preCore/pregenomic RNAs, the surface protein RNAs PreS1 and PreS2/S, and the HBx RNA, that share not only the same 3' end but also at different degree a larger part of their sequences making difficult to quantify HBV RNA species individually.The lack of an effective quantitative approach of the different viral RNAs remains a major hurdle in the investigation of different aspects of the viral life cycle, from basic knowledge of the interaction between HBV and its host throughout infection or at the different stages of the chronic hepatitis disease, to the impact of drugs on HBV transcription and viral RNAs metabolism.

To overcome this difficulty, we developed a method based on multiplexed Droplet Digital PCR to detect and quantify specifically and simultanously all the HBV RNA species including splice variants. We applied this technique to measure variations of viral RNA transcripts in different cellular models of HBV replication / expression (HepG2-NTCP cells, HepAD38 cells and primary human hepatocytes). Our method allowed us to study the kinetics of HBV RNAs expressions at very early time after infection when transcription is very low and to show that the expression of the different HBV RNAs is differentially regulated over time, a mechanism that allow the establishment of an efficient cccDNA transcription.

Oral presentation

## Enter the Multi-Omics Era with BGI Genomics: Technical Highlights

**Ruoyang Zhai**

*BGI, France*

BGI Genomics is one of the world's leading providers of integrated sequencing and mass spectrometry services, specializing in genomics, high-resolution transcriptomics, single-cell omics, spatial omics, epigenomics, proteomics, metabolomics, and multi-omics. With over 20 years of experience, BGI Genomics empowers researchers to accelerate discovery by delivering rapid, high-quality results through a comprehensive portfolio of cost-effective, cutting-edge technologies. At RNAOcc', we are pleased to present highlights of our services with patented innovations in single-cell RNA sequencing, high-resolution spatial transcriptomics, and long-read sequencing, including: 1) DNBelab C Series 4: high-throughput single-cell RNA sequencing solution; 2) Stereo-seq: True single-cell resolution spatial transcriptomics solution with whole transcriptome profiling and; 3) CycloneSEQ ER: Patented long-read sequencing technology.

Oral presentation

# The RNA binding activity of the Drosophila Hox Transcription Factor Ultrabithorax contributes to muscle morphogenesis in developing embryos

**Wan Xiang[1,2,3], Constanza Blanco[3], Panagiotis Boumpas[3,4], Maily Scorceletti[3,4], Ashley Suraj Hermon[3], Samir Merabet[3], Julie Carnesecchi[1*]**

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Transcription factors (TFs) orchestrate tissue development and maintenance by controlling different molecular layers of gene programs. Beyond their function on DNA, TFs also interact with RNA and regulate mRNA splicing. Yet, a large gap remains in understanding TF-RNA regulatory function and its significance for tissue morphogenesis. Previous work in our lab demonstrated that the Hox TF Ultrabithorax (Ubx) binds RNA and regulates alternative splicing. Moreover, Ubx genetically interacts with several splicing factors including the small nuclear ribonucleoprotein snRNPU1-70K to regulate embryonic muscle development. Yet, the significance of Ubx-RNA interaction in morphogenesis is unknown. My PhD project investigates this issue. Relying on Ubx/snRNPU1-70K genetic interaction in somatic musculature of Drosophila embryos, we assessed the ability of different DNA/RNA binding Ubx mutants to rescue the muscle alteration. Moreover, we evaluated the global function of Ubx DNA/RNA binding mutants in the Ubx null homozygous mutant context. This indicates that Ubx-RNA binding activity contributes to its homeotic function. Presently, I am investigating Ubx-splicing function in vivo in muscle cells by employing single-molecule fluorescence in situ hybridization (smFISH) to detect the expression of exon RNAs differentially spliced by Ubx. Altogether, these results uncovered the significance of Ubx-RNA activity in myogenesis. In future, this work will provide new insight into the contribution of Ubx-splicing function in muscle morphogenesis.

Oral presentation

# Transcriptional Regulation Mechanisms Mediated by m6A RNA Modification in Drosophila melanogaster

**Margot Lugoboni, Paul Terzian, Yoan Renaud, Guillaume Junion**

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Gene expression is controlled through a multi-layered regulatory process. Transcription, the first critical step, is shaped by the chromatin environment, which facilitates transcription factor recruitment leading to its initiation. However, over five decades ago, researchers discovered a transcription regulatory checkpoint termed RNA polymerase II (RNAPII) pausing. Shortly after transcription initiation, RNAPII undergoes a transient transcriptional arrest near promoters, called "pausing." Our team previously demonstrated that $m^6A$ methylation on nascent transcripts plays a role in releasing paused RNAPII.
My thesis investigates the molecular mechanisms by which the $m^6A$ epitranscriptomic mark promotes RNAPII pause release. We hypothesized that R-loops, a three-stranded nucleic acid structures, act as key intermediaries in this process. Using BisMapR-seq, a high-resolution genomic approach in *Drosophila*, we mapped R-loop landscapes and assessed their relationship with $m^6A$. We have now validated that R-loops predominantly localize to promoters and are enriched at active en- hancers and that R-loop levels correlate with both $m^6A$ methyltransferase complexes and $m^6A$-marked RNA at promoters. To dissect the transcriptional consequences of R-loops, we are now performing RNAPII CUT&RUN to profile transcriptional dynamics after modulating R-loop regulators (e.g., RNase H, helicases). This integrative approach aims to resolve how R-loops and $m^6A$ collaboratively regulate RNAPII progression, offering new insights into gene expression control

Oral presentation

# Long-read sequencing identifies full-length circular RNAs linked with therapy resistance in pediatric cancers

**Chloé Bessiere[1], Elissa Andraos[1], Loélia Babin[1], Johannes Markus Riepl[2], Suzanne Turner[3], Jamie Mathews[3], Annabell Szymansky[2], Marco Lodrini[2], Hedwig Elisabeth Deubzer[2], Angelika Eggert[2], Charlotte Rigaud[4], Véronique Vérgé[5], Johannes Hubertus Schulte[6], Laurence Lamant[7], Fabienne Meggetto[1], Christine Gaspin[8], Steffen Eberhard Fuchs[1]**

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**Background**: Treatment resistance remains a source of cancer-related deaths. Resistance can develop to targeted and chemotherapy drugs. ALK inhibitors (ALKi) are added to treatment for Anaplastic Large Cell Lymphoma harboring ALK translocations (ALK+ ALCL) and treatment for another childhood cancer, neuroblastoma with ALK genomic aberrations. Complex RNA variants, circular RNAs (circRNAs) and dysregulated alternative splicing have been associated with resistance formation, especially important in tumors with low mutational burden, such as pediatric cancers [(1)]. Emerging as potential biomarker candidates are the noncoding circRNAs, which are highly stable and can be detected in body fluids.
**Aims**: We aimed to identify circRNA isoforms associated with therapy resistance in liquid biopsies from children with ALK-driven cancers that could act as surrogate biomarkers for resistance.
**Methods**: We adapted a long-read sequencing approach using the Oxford Nanopore platform to characterize full-length circRNA isoforms. A preclinical model platform sensitive or resistant to ALKi was created to assess circRNA association with resistance. Liquid biopsies from patients sensitive or resistant to ALKi were analyzed.
**Results**: Nanopore sequencing in ALCL ALKi-resistant preclinical models and two different detection tools (for higher confidence) identified 9,702 full-length circRNAs. Three circRNAs were specifically upregulated in resistant models. ALCL ALKi-resistant models showed higher circRNA diversity, indicated by an increased length and more alternative splicing events. Using patient- derived xenograft models and a cohort of ALK+ ALCL patients, two of the circRNA variant candidates were associated with worse outcomes. Similar findings were obtained when exploring a unique collection of blood plasma samples from patients with neuroblastoma, with the two circRNA variants more abundant in ALKi-resistant cases. High expression of these circRNA candidates in tumor biopsies was associated with shorter event-free survival.
**Conclusions**: Our study demonstrates that specific full-length sequencing allows identifying disease-relevant circRNA variants with promise for development as clinical biomarkers for patient monitoring during disease course and treatment.

*[1] Tan WQ, Yuan L, Wu XY, He CG, Zhu SC, Ye M. Exosome-delivered circular RNA DLGAP4 induces chemoresistance via miR-143-HK2 axis in neuroblastoma. Cancer Biomark. 2022;34(3):375-384.*

Oral presentation

## An issue of translation: gene regulation mechanisms to unlock neuron regeneration

**Julia Schaeffer[1,5], Noemie Vilallongue[1], Charlotte Decourt[1], Beatrice Blot[1], Nacera El Bakdouri[1], Elise Plissonnier[1], Blandine Excoffier[1], Antoine Paccard[1], Jean-Jacques Diaz[2,3,4], Sandrine Humbert[1], Frederic Catez[2,3,4], Frederic Saudou[1], Homaira Nawabi[1], Stephane Belin[1]**

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How individual cells organize and maintain distinct intracellular compartments is a central question in Cell Biology, as it underpins their structure, function, communication within a tissue and homeostasis. Neurons are a stereotypical examples of cells highly polarized and organized into structurally and functionally distinct sub-domains, including the axon responsible to emit electrical signals to their target cells.

In adult mammals, central nervous system (CNS) neurons fail to regenerate their axon after a lesion, hindering functional reconnection. Efforts to address this regeneration failure have focused on the growth-inhibitory role of the environment[1] and on properties of adult neurons themselves[2]. Currently, the best strategy to promote regrowth is to activate injury- or developmentally- downregulated pathways that regulate growth and metabolism, such as the mTOR pathway.

Our recent work has identified the translation of specific mRNAs into proteins as an active layer of gene regulation in mTOR-induced axon regrowth, whereby the translational complex selectively translates a specific subset of regrowth-associated mRNAs regardless of their transcriptional state[3]. This selectivity is controlled by the customization of ribosomes with specific factors, among which we identified the protein Huntingtin[3]. Our results challenge the view that translation is a passive process in gene regulation and contribute to bridging gene expression and axon regrowth capacity[4].

Yet, despite advancing our understanding of regeneration mechanisms, the manipulation of selectively translated mRNAs is not sufficient to promote axon regrowth. Besides, current regeneration models suffer from a limited efficacy and may have multiple untargeted effects with oncogenic risks, preventing their therapeutic application. Thus, we need to better understand how gene expression is regulated in regenerating neurons and notably in the axon compartment itself.

Indeed, it is now established that gene regulation is spatially compartmentalized in neurons and that axons behave autonomously from the soma thanks to a distinct gene regulatory program. For this, axons rely on local translation, a process critical for its guidance, maintenance and function. However, very little is known about local translation in the context of CNS injury and regeneration.

Here, I will highlight how the translational complex regulates axon regrowth, supporting growing evidence that translation shapes development, cell type specification and stress responses. I will also present the rationale for exploring local translation in injured axons as a key for their regrowth and reconnection[5]. I will discuss the working hypothesis that the intrinsic regrowth capacity of an axon depends on local selective translation of a specific subset of mRNAs and on local supply of ribosomes, and that these processes are defective in non-regenerative systems such as injured CNS neurons. This approach can overcome the limitations of soma-focused profiling and of current regeneration strategies that have limited or side effects, thus offering a new perspective to understand the biology of the axon compartment: how it is specified, how it is maintained and how it responds to injury.

1. *Schaeffer, J., Vilallongue, N., Belin, S., and Nawabi, H. (2023). Axon guidance in regeneration of the mature central nervous system: step by step. Neural Regen Res 18, 2665–2666. https://doi.org/10.4103/1673-5374.373663.*
2. *Mahar, M., and Cavalli, V. (2018). Intrinsic mechanisms of neuronal axon regeneration. Nat Rev Neurosci 19, 323–337. https://doi.org/10.1038/s41583-018-0001-8.*
3. *Schaeffer, J., Vilallongue, N., Decourt, C., Blot, B., Bakdouri, N.E., Plissonnier, E., Excoffier, B., Paccard, A., Diaz, J.-J., Humbert, S., et al. (2023). Customization of the translational complex regulates mRNA-specific translation to control CNS regeneration. Neuron 111, 2881-2898.e12. https://doi.org/10.1016/j.neuron.2023.06.005.*
4. *Schaeffer, J., and Belin, S. (2024). Axon regeneration: an issue of translation. Comptes Rendus. Biologies 347, 249–258. https://doi.org/10.5802/crbiol.169.*
5. *Schaeffer, J., and Belin, S. (2021). Axonal protein synthesis in central nervous system regeneration: is building an axon a local matter? Neural Regen Res 17, 987–988. https://doi.org/10.4103/1673-5374.324835.*

Oral presentation

## Understanding the epitranscriptomic landscape regulated by the p53 pathway

**Javier Florido[1], Oriana Villafraz[1], Michela Di Michele[1], Aurore Attina[2], Sonia Garcia- Llorens[2], Pierre-François Roux[1], Geneviève Rodier[1], Virginie Marchand[3], Matthieu Lacroix[1], Lourii Motorine[3], Alexandre David[2], Laurent Le Cam[1]**

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Our laboratory is interested in understanding how deregulation of the p53 tumor suppressor pathway impinges on gene expression at both the transcriptional and post-transcriptional levels. Over the past 10 years, we generated one of the largest collections of genetically engineered mouse models (more than 20) harboring conditional knock-out and knock-in alleles for key components of the p53 pathway (p53, Mutp53, Mdm2, Mdm4, E4f1). An extensive multi-omic (RNAseq, proteomic, metabolomic, epitranscriptomic) characterization of primary cells isolated from these mouse models highlighted unexpected links between p53 (or some of its regulators) and the epitranscriptome. One of these connections involves E4F1, a multifunctional protein initially characterized as a transcription factor but which also display an atypical E3 ligase activity targeting p53. Here, I will show our latest results showing how E4F1 controls the expression of several genes encoding enzymes regulating RNA modifications, including Nsun5 and Elp3, which target rRNA/mRNA methylation (5-methylcytosine or m5C) and tRNAs acetylation (at the wobble position uridine 34 (U34)), respectively. Using mice lacking E4F1 in the central nervous system, we show that E4F1-mediated control of the epitranscriptome is implicated in the regulation of translation fidelity during neuronal development.

*[1] Di Michele M, Attina A, Roux PF, Tabet I, Laguesse S, Florido J, …, Lacroix M, Le Cam L. E4F1 coordinates pyruvate metabolism and the activity of the elongator complex to ensure translation fidelity during brain development. Nat Commun. 2025 Jan 2;16(1):67. doi: 10.1038/s41467-024-55444-y.*

Oral presentation

# A lncRNA-RNase mechanism at the center of the elimination process of nucleolus-related inclusions

**Le Tareau Léo[1], Guette Charlotte[2], Trauchessec Hélène[1], O'Donohue Marie-Françoise[2], Gleizes Pierre-Emmanuel[2], Bousquet Marie Pierre[3], Xirodimas Dimitris[1]**

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RNA molecules are key scaffolding elements that drive phase separation in many biomolecular condensates. A prime example is the nucleolus, which forms through liquid–liquid phase separation, is organized around ribosomal DNA, and contains a large proportion of ribosomal RNA (rRNA). Under proteotoxic stress—such as heat shock, acidosis, or proteasome inhibition—the nucleolus undergoes a liquid-to-solid phase transition: rRNA transcription halts, and misfolded proteins accumulate, forming nucleolus-related inclusions (NoRIs).

Our studies revealed that under such stress conditions where rRNA production stops, a specific region of the rDNA intergenic spacer is transcribed, producing a group of long non-coding RNA (IGS lncRNA). We found that IGS lncRNAs accumulate specifically within the stress-induced NoRIs and that control the clearance of NoRIs.

Proteomic analyses and live-cell imaging during both the formation and clearance of NoRIs revealed a highly organized system: a specific RNase is sequestered within the solid phase early during stress. Upon stress relief, the RNase is released, leading to degradation of the IGS lncRNA that increases the solubility of NoRIs. This in turn activates the ubiquitin–proteasome system (UPS), which facilitates refolding or degradation of misfolded proteins, enabling nucleolar recovery.

Our studies have uncovered a central role of a novel class of lncRNAs in aggregate management. In co-ordination with the UPS system, IGS lncRNAs allow the efficient clearance of stress-induced nuclear aggregates.

Oral presentation

# Deciphering the translation dynamics of SARS-CoV-2 UTRs

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic, represents a significant global public health threat. SARS-CoV-2 is a positive-strand, RNA-enveloped virus belonging to the Betacoronaviridae family. It primarily targets human lung cells and can cause severe respiratory disease.[1] SARS-CoV-2 viral particle mainly consists of structural proteins and a ribonucleoprotein complex housing the 30 kb genomic RNA.[2] During the infection, SARS-CoV-2 genomic RNA (gRNA) transcription and replication occurs within Double-Membrane Vesicles (DMVs) derived from the endoplasmic reticulum.[3] After transcription, the gRNA exits these vesicles and is packaged into new viral particles during the assembly phase.[4] Additionally, SARS-CoV-2 employs a discontinuous transcription mechanism, generating subgenomic RNAs (sgRNAs) that encode structural and accessory proteins. This intricate process produces a pool of sgRNAs, each containing a common 75-nucleotide leader sequence and a variable region in their 5' untranslated regions (UTRs). SARS-CoV-2 generates up to 10 canonical sgRNAs alongside its gRNA.[5]

This project aims to quantitatively characterize the molecular and cellular mechanisms governing the translation of viral RNAs. It specifically investigates the roles of different 5' UTRs in the translation of both genomic and subgenomic RNAs, aiming to uncover the spatiotemporal coordination of viral protein synthesis. Real-time monitoring of reporter viral RNA localization and translation kinetics in living cells will be achieved using antibody based live imaging of nascent peptide systems[6] Followed by Fluorescence Correlation Spectroscopy (FCS) and single-RNA tracking.

We have shown that the SARS-CoV-2 5'UTRs are highly conserved among variants suggesting an important role of the 5'UTRs in sgRNA translation. We have also shown that variability in the 5'UTR has an impact on the translation kinetics of SARS-CoV-2 RNAs correlated with the amount of translated protein. Altogether our data suggest a strategic advantage of the variable sequence embedded into the different 5'UTRs to regulate SARS-CoV-2 replication.

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Oral presentation

## Epstein-Barr virus v-snoRNA1 modulates translation of the infected host cell

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The genome of the Epstein-Barr virus encodes a small nucleolar RNA, v-snoRNA1, whose function has remained elusive (Hutzinger *et al*. 2009, PLoS Pathog. PMID:19680535). Recently, we found that v-snoRNA1 is a 2-*O*-methylation guide of the human 18S and 28S ribosomal RNAs and induces post-transcriptional modifications in two functionally important regions of the ribosome. Homologues of v-snoRNA1 are also found in five other herpesviruses hinting at an evolutionary advantage of ribosomal RNA modification.
Functional analysis revealed that human ribosomes carrying the two post-translational RNA modifications guided by v-snoRNA1 are translationally less competent and more error-prone. However, the effect of this viral translational modulation is not the same on all mRNAs and this creates the possibility of differential translation. As an example, we demonstrate differential translation of an Epstein-Barr virus protein playing a crucial role in viral latency establishment. To assess the biological consequences of v-snoRNA1-induced translational modulation, we used a B cell line with an integrated Epstein-Barr genome. In this cell line, we specifically knocked out the v- snoRNA1 gene. As an additional control, we generated another cell line by reintroducing the v- snoRNA1 gene into the knock-out B cells by stable transfection. In the resulted cell lines, we analyzed expression of both host and Epstein-Barr virus genes at translational and transcriptional levels. Many v-snoRNA1-related changes in host gene expression can be linked to signaling and immune response. This raises the possibility that manipulation of the host translational machinery might contribute to the lymphoblastoid transformation of the infected B cells.

Oral presentation

## Microbial metabolite preQ1 regulates host cell physiology through selective tRNA degradation and translation regulation

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Microbial metabolites are well-known regulators of host physiology. One such metabolite, queuine, is incorporated at the wobble position of specific host tRNAs, where it contributes to translational regulation and tRNA stability. In the queuine biosynthesis pathway, microbes also produce the precursor metabolite pre-queuosine1 (preQ1). However, its effects on host cell biology and the underlying mechanisms remain largely unexplored. Here, we show that preQ1 significantly inhibits proliferation in both human and mouse cells. This effect is suppressed or reversed by queuine and is dependent on the host tRNA-guanine transglycosylase (TGT) enzyme responsible for queuine incorporation. Using both in vitro cell culture systems and in vivo mice models, we demonstrate that preQ1 is bioavailable in plasma and tissues and is efficiently incorporated into host tRNAs. Importantly, preQ1 treatment suppresses tumour growth in a mouse cancer xenograft model. Mechanistically, preQ1 selectively reduces the levels of its cognate tRNAs, leading to a codon- dependent repression of translation, particularly of housekeeping genes. We identify the inositol-requiring enzyme 1 (IRE1) ribonuclease as the key enzyme mediating the selective degradation of preQ1-modified tRNAs on translating ribosomes. Collectively, our findings reveal a previously unrecognized microbial-host interaction in which preQ1 modulates tRNA stability and translation, thereby influencing cellular proliferation and tumour progression. These results highlight preQ1 as a novel microbial effector molecule with potential implications in cancer biology and host-microbe metabolic crosstalk.

Oral presentation

## mRNA degradation as a rescue mechanism for translation-inhibiting antibiotics

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The efficacy of translation-inhibiting antibiotics, such as chloramphenicol, depends not only on their ability to stall elongating ribosomes on mRNA but also on the cell's intrinsic mechanisms to alleviate these effects. Traditional models of translation inhibition often overlook the impact of mRNA turnover on antibiotic susceptibility. Notably, the typical bacterial mRNA lifetime (1–5 minutes) [Balakrishnan et al., 2022] is comparable to the timescale of antibiotic dissociation from ribosomes, with chloramphenicol unbinding occurring on the order of 12 minutes [Harvey et al., 1980]. This overlap in timescales highlights the need to account for average mRNA lifetime when modeling the cellular response to translation-inhibiting antibiotics.

Here, we present a combined theoretical and experimental approach to quantify how mRNA degradation influences translation rates under antibiotic stress. We develop a stochastic model that explicitly incorporates both ribosome pausing by antibiotics and mRNA degradation, allowing us to predict protein production as a function of antibiotic concentration and mRNA turnover. To test these predictions, we analyze *E. coli* mutants lacking the major 3'-5' exonucleases RNase II and PNPase, revealing how impaired mRNA degradation increases cellular susceptibility to chloramphenicol.

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Poster presentation

# Exploring RNA destabilization mechanisms in biomolecular condensates through atomistic simulations

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Biomolecular condensates are currently recognized to play a key role in organizing cellular space and in orchestrating biochemical processes. Despite an increasing interest in characterizing their internal organization at the molecular scale, not much is known about how the densely crowded environment within these condensates affects the structural properties of recruited macromolecules. Here we adopted explicit-solvent all-atom simulations based on a combination of enhanced sampling approaches to investigate how the conformational ensemble of an RNA hairpin is reshaped in a highly-concentrated peptide solution that mimics the interior of a biomolecular condensates. Our simulations indicate that RNA structure is greatly perturbed by this distinctive physico-chemical environment, which weakens RNA secondary structure and promotes extended non-native conformations.
The resulting high-resolution picture reveals that RNA unfolding is driven by the effective solvation of nucleobases through hydrogen bonding and stacking interactions with surrounding peptides. This solvent effect can be modulated by the aminoacid composition of the model condensate as proven by the differential RNA behaviour observed in the case of arginine-rich and lysine-rich peptides.

*M. Boccalini, Y. Berezovska, G. Bussi, M. Paloni, & A. Barducci, Exploring RNA destabilization mechanisms in biomolecular condensates through atomistic simulations, Proc. Natl. Acad. Sci. U.S.A. 122 15 (2025).*

Poster presentation

## Unraveling the stress response molecular mechanism of an imprinted p53-associated tumor suppressor lncRNA

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Meg3 is a nuclear lncRNA that is transcribed from a large ncRNA polycistron at the Dlk1-Dio3 domain, from the maternal chromosome only. This imprinted locus plays diverse roles in development and disease and is a regulator of neurodevelopment. Previously, our lab showed how Meg3 lncRNA prevents in cis the upregulation of the Dlk1 gene, which encodes an antagonistic ligand of Notch (Sanli I. et al., Cell Reports, 2018; Farhadova S. et al., Nucleic Acids Research, 2024). However, little is known about the putative roles in trans of this conserved lncRNA.

We find that during neural differentiation of murine embryonic stem cells (mESCs), the nuclear localization of Meg3 shifts from a single cis focus (in mESCs) to multiple trans foci (differentiated cells). Using magnet-activated cell sorting to purify neurons, and RNA-FISH coupled to immunofluorescence, we find that the multiple trans foci form specifically in neurons. Our preliminary data also show a partial co-localization of the foci with nuclear bodies that are linked to stress responses. Testing different kinds of stress, we find that neurons -and not other neural cell types- significantly increase Meg3 RNA levels upon low-dosage treatment with a genotoxic agent. Interestingly, members of our lab showed previously that transient overexpression of MEG3 activates a subset of genes of the p53 pathway in human cancer cells. Importantly, this previous work pinpointed the structural features of the human MEG3 that are required for the p53 pathway activation (Uroda T. et al., Molecular Cell, 2019). We are currently exploring whether these features are conserved in the mouse lncRNA as well. To functionally study the stress-related Meg3 responses in neurons, we generated by CRISPR-Cas9 a set of mESC cell lines with different deletions in the Meg3 polycistron. In addition, to gain a deeper understanding of the pathways influenced by Meg3 during stress responses, we are performing RNA hybridization capture experiments to identify putative lncRNA-interacting proteins. These will be validated by RNA- protein immunoprecipitation, and by dedicated functional assays. In summary, for the first time our study investigates the mechanisms through which a conserved imprinted lncRNA acts in post- mitotic cells (neurons) and following DNA damage induction.

Poster presentation

# Unveil regulation of RNA-binding protein Tristetraprolin mRNA in Cystic Fibrosis: a new way for inflammation resolution

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## Objectives

Cystic fibrosis (CF) displays an inflammatory context specifically in bronchial epithelia where some RNA-Binding proteins (RNA-BP) show a deregulated expression. Tristetraprolin (TTP) is an anti-inflammatory RNA-BP, that destabilizes mRNA of pro-inflammatory proteins, participating in resolution of inflammation. We now assess efficacy of a new therapy approach based on stabilizer oligonucleotides targeting 3'UTR mRNAs to increase the TTP mRNA level for modulating inflammatory response in CF.

## Materials and methods

Efficacy of TTP overexpression has been evaluated by either CRISPR activation (CRISPRa) assay or an oligonucleotide-based strategy. The effect of oligonucleotides on TTP 3'UTR mRNA has been evaluated by luciferase activity where luciferase cDNA is followed by TTP 3'UTR part in Beas-2B cells. The effect of oligonucleotides was further confirmed by mRNA and protein level quantification as well as ELISA dosage in non-CF bronchial, primary CF and THP-1 cells.

## Results

Overexpression of TTP by CRISPRa induced an increase in TTP mRNA and a reduction of proinflammatory cytokines mRNA level, suggesting that TTP mRNA level elevation led to inflammation resolution in CF context.

We next designed oligonucleotides that stabilize TTP mRNA expression. To optimize oligonucleotide efficacy, we compared different different chemistry for the oligonucleotides design, and delivery ways. Introduction of TSB showed an increase in protein TTP level in CF cells and macrophages. In inflammatory context, dosage of CF and M1-derived THP-1 cells supernatants showed a decrease in pro-inflammatory cytokines secretion.

## Conclusion

Conceive an oligonucleotide strategy permits to target the 3'UTR regulatory part of the RBP TTP mRNA and led to stabilize TTP protein level associated with the cytokines secretion decrease in both CF and macrophages cells.

Poster presentation

## Interplay by Condensation and RNA Binding Through a Minimal Model

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We investigate the properties of protein-RNA condensates with a novel ultra coarse-grained model capable of simulating real sized membraneless organelles. Our model relies on two energetic parameters, protein-protein and protein-RNA interactions, and enables an exhaustive exploration of condensate behaviour by systematically variation of RNA concentration and interaction patterns.
Despite its simplicity, this model can predict key features of biomolecular condensates. First, we observe entropy-driven re-entrant phase separation with RNA excess, a hallmark of coacervate systems. Additionally, our results show that the condensate environment enhances protein–RNA binding, highlighting the role of protein localization within the droplet. Finally, simulations involving heterogeneous components revealed the emergence of complex, multiphase architectures with distinct binding properties.

Poster presentation

## Imaging HIV-1 transcription to understand viral latency

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Combinatorial antiretroviral therapy controls HIV infections. However, therapy interruption leads to viral rebounds provoked by the reactivation of latently infected cells. Current data suggest that latency exit is stochastic, reflecting the random fluctuations of viral transcription[1,2]. However, while the rate at which latent cells become reactivated is a key clinal variable, viral transcription in latent cells is not well characterized. Here, we developed an MS2 tagged dual color HIV-1 vector, enabling both the selection of latently infected cells and live cell imaging of viral transcription with single molecule sensitivity[3,4]. Viral transcription was imaged in a latent T cell clone carrying an intact viral genome under basal conditions and upon activation. Under basal conditions, latent cells exhibited rare bursts of transcriptional activity. Each burst produced only few RNAs and were separated by inactive periods lasting hours, highlighting the very low transcriptional activity of latent viruses. Latent cells displayed significant cell to cell transcriptional heterogeneity and basal viral transcription was insufficient to trigger spontaneous latency exit.

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Poster presentation

## Identification of Aub partners for the regulation of Drosophila germline stem cell fate

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Metabolic pathways play a crucial role in stem cell fate. While glycolysis promotes stem cell renewal, a switch to oxidative phosphorylation contributes to stem cell differentiation. To understand the underlying mechanism, we use *Drosophila* female germline stem cells (GSCs). In ovaries, oogenesis starts in a structure called the germarium that contains two to three GSCs. Each GSC divides asymmetrically to produces one daughter cell that remains stem cell by self-renewal, while the other differentiates and produces a germline cyst.
The lab previously showed that energy metabolism contributes to the fate of GSCs in *Drosophila* ovaries. This regulation is mediated by the PIWI protein, Aubergine (Aub), an Argonaute protein that target mRNAs through piRNAs (PIWI-interacting RNAs). Aub is able to bind glycolytic mRNAs, in a piRNA-dependent manner and activates their translation in GSCs. The loss of Aub leads to a metabolic switch towards oxidative phosphorylation and induces GSC differentiation.
Aub and glycolytic mRNAs are expressed in both stem cells and differentiating cysts throughout the germarium, indicating the requirement of Aub co-regulators to ensure a tight mRNA regulation between stem cells and differentiating cells. Motif analyses within 60 nucleotides of Aub binding sites on glycolytic mRNAs revealed potential RNA-binding proteins that might act as Aub coregulators. Two of them, IGF-II mRNA binding protein (Imp) and Syncrip (Syp) proteins were known for their role in translation regulation in neuroblasts. Imp and Syp are expressed in opposite temporal gradients that specify neuronal stem cell fates during larval/pupal development. Moreover, iCLIP experiments performed in larval brain identified binding sites of Imp and Syp in metabolic mRNAs (Lee et al. 2025 Science Advances).
Interestingly, we found that Imp is highly expressed in GSCs, and *Imp* knock-down leads to a GSCs loss, consistent with a role of Imp in GSC self-renewal. Conversely, clonal analysis shows that *syp* mutant germline clones produce tumors of GSCs-like cells that are unable to differentiate, implying a potential role of Syp in stem cell differentiation. In addition, Imp and Aub co-immunoprecipitate in ovaries in agreement with a potential role of Imp with Aub for glycolytic mRNA regulation in GSCs.
Taken together, these preliminary data suggest that, as in neuroblasts, Imp and Syp may play opposite functions in regulating stem cell fate in *Drosophila* ovaries, Imp controlling GSC maintenance and Syp their differentiation.

Poster presentation

## Co-translational determination of quaternary structures in chaperone factories

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Protein complexes and non-coding RNPs are complex macromolecular assemblies that require specific factors for their assembly. These factors have been shown to chaperone unassembled subunits, improve efficiency and specificity of the assembly process, transport subunits across the cell and perform quality controls[1,2]. Recent studies suggested that the assembly of multi-subunit complexes is intimately coupled with translation, with many observations converging to the ribosome, indicating that it may function as a hub driving assembly[3,4]. The HSP90/R2TP quaternary chaperone assembles key cellular machines[6], including the three nuclear RNA polymerases[7] and many non-coding RNPs such as the box C/D and H/ACA snoRNPs[2] and U4 and U5 spliceosomal snRNPs[7]. Here, we characterized the RNA associated to R2TP and found that it binds many partners co-translationally. Its co-translational interactome further reveals many novel potential clients and identifies clients bound only co-translationally, only post-translationally, or both. For pairs of subunits assembling together and bound co-translationally by R2TP, only a marginal proportion of their mRNAs is co-localized and co-translated. Instead, the HSP90 and R2TP chaperones induce the formation of condensates accumulating client mRNAs and thus favouring co-translational interactions between chaperones and clients. The R2TP then cycles between co- and post-translational steps and this is regulated by ATP: it binds co-translationally in absence of ATP and becomes released from post-translational assembly intermediates by ATP hydrolysis. Assembly of protein complexes is thus initiated early by chaperones and this mechanism, dubbed co-translational chaperone channelling (cha-cha), substitutes for the rarity of co-localized/co-translated mRNAs.

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Poster presentation

# Biochemical and Structural Characterization of the NCBP3–CBC–EJC Axis in mRNA Export

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In eukaryotic gene expression, RNA polymerase II transcribes DNA into pre-mRNA, which undergoes a series of maturation steps including 5′ capping, splicing, and 3′ polyadenylation. During splicing, the Exon Junction Complex (EJC) comprising EIF4A3, Y14, MAGOH, and MLN51—is deposited 20–24 nucleotides upstream of exon-exon junctions. Mature mRNA is then exported into the cytoplasm, in a process tightly coordinated by a network of RNA-binding proteins. The Cap-Binding Complex (CBC), a heterodimer of CBP20 and CBP80, binds the 5′ cap structure of nascent transcripts (1,2) and, via ARS2, forms the CBCA platform (3). CBCA orchestrates RNA biogenesis (4) through mutually exclusive interactions with RNA effectors such as PHAX (5,6) (for snRNA export), ZC3H18 (6) (for RNA degradation), and NCBP3 (7) (for mRNA export). NCBP3 has been identified as a potential nuclear export regulator, interacting with EJC (8,9), though its precise mode of action remains unclear. The aim of my project is to biochemically and structurally characterize the molecular interplay among NCBP3, CBC and EJC. Using *in vitro* protein-protein interaction assays and structural approaches such as cryo- electron microscopy, this work will provide mechanistic insights into the coordination of mRNA processing and export.

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Poster presentation

## 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 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.

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