IMCBio symposium

The symposium is taking place from the 18th to the 22th of November 2024. You will be able to listen to presentations about various subjects in biology. Directly after their presentations, or during breaks, you will be able – and are encouraged- to ask questions to the speakers.

You can find the program on the next pages.

Monday 18/11- Salle Léon Hirth IBMP

Plants and Virology

9h00-10h00– **Philip Carella** (John Innes Centre, United-Kingdom)

Online presentation via Zoom

Connection link: https://us06web.zoom.us/j/81281928071?pwd=IfLxuwpEI3qn89ZaXvLrlbavb6laYD.1

ID : 812 8192 8071
Code : 239335

Functional conservation of NLR immune receptors on a macroevolutionary timescale

Chair: Ikram Bendraoua & Jérémy Dufat

Nucleotide-binding domain and leucine-rich repeat (NLR) proteins are a prominent class of intracellular immune receptors in plants. However, our understanding of plant NLR structure and function is limited to the evolutionarily young flowering plant clade. In this work, we describe an extended spectrum of NLR diversity across divergent plant lineages and demonstrate the structural and functional similarities of N-terminal immune domains that trigger common responses in distantly related land plants.

10h00-10h30: Coffee break – Hall IBMP

10h30-11h30 – **Thomas Michiels** (de Duve Institute UCLouvain, Belgium)

Use of short linear motif (SLiM) mimicry by viruses to hijack cellular pathways and escape innate immunity

Chair: Bruno Del Carpio Martinez & Medhi Krajkovic

Short linear motifs (SLiMs) are 3-to-10 amino acid-long protein motifs, typically found in disordered protein regions, which nucleate protein-protein interactions by interacting with structured regions of other proteins. SLiMs usually mediate low-affinity, transient and dynamic interactions and are therefore considered to play a critical role in regulating signal transduction pathways to maintain cell homeostasis.

Identification of SLiMs used to be sporadic and based on low-throughput methods such as co-immunoprecipitation coupled to mass spectrometry. Yet, such methods enabled the identification of a number of SLiMs, which are listed in the ELM database [1].

More recently, identification of SLiMs has been boosted by high-throughput interactomic methods, such as phage display [2]. Nowadays the ELM database contains more than 4000 entries. Importantly, many AI-based (mostly based on alphaFold) methods are currently developed to predict SLiM-based protein-protein interactions. It is anticipated that hundreds of thousands of such interactions occur in cells.

Given their very small size, SLiMs easily appear through sequence evolution, particularly in fast replicating organisms such as viruses. We previously showed that highly divergent pathogens, including viruses and bacteria, evolved in a convergent fashion to hijack cellular kinases of the RSK family [3]. Proteins like the leader (L) protein of Cardioviruses (picornaviruses), ORF45 of Kaposi sarcoma associated herpes virus (HHV-8 or KSHV), or YopM from *Yersinia* species, do all carry a similar DDVF-like motif that enables them to interact with a loop and a hydrophobic pocket formed at the level of the KAKLGM sequence at the surface of RSK kinases.

Remarkable conservation of the SLiM docking site in RSKs suggested a physiological role for this site. Using SLiM prediction tools and AlphaFold docking, we screened the human proteome for proteins that would interact with RSKs through a DDVF-like SLiM. Next, using co-immunoprecipitation experiments, we showed that two candidates previously known as RSK partners, FGFR1 and SPRED2, as well as two candidates identified as novel RSK partners, GAB3 and CNKSR2 do interact with RSKs through a similar interface as the one used by pathogens [4]. Interestingly, all these proteins do belong to the well-known RAS-ERK MAPK pathway, which regulates important functions of the cell, suggesting that pathogens evolved to hijack this MAPK pathway by "SLiM mimicry".

In the case of Theiler's murine encephalomyelitis virus (TMEV, a cardiovirus), recruitment of RSK through the DDVF motif of the leader protein (L) was shown to be instrumental in the perturbation of nucleo-cytoplasmic trafficking of host proteins triggered by the virus [5].

Using the "analog-sensitive kinase" strategy developed in the laboratory of Kevan Shokat [6], we showed that, when complexed to L, RSK was redirected toward the nuclear envelope where RSK phosphorylated non-conventional substrates such as FG-nucleoporins, leading to the disruption of the nuclear pore complex [5].

Recently we identified the motif in the L protein, which is responsible for targeting the L-RSK complex toward the nuclear pore. Interestingly, this motif corresponds to a SLiM that is shared by proteins from other pathogens such as SARS-CoV-2, KSHV or vesicular stomatitis virus. It is still unclear whether this SLiM also mimics the SLiM of cellular proteins that regulate the nucleo-cytoplasmic traffic.

Thus, the leader protein of TMEV uses a combination of two SLiMs to retarget cellular kinases toward unconventional substrates, and thereby to compromise nucleo-cytoplasmic trafficking in the cell. Interestingly, both SLiMs are shared by other pathogens and may have arisen independently by by SLiM mimicry.

It is likely that the fast development of AI-based prediction tools and of high-throughput interactomic methods will document how combinations of SLiMs are used to fine tune signaling processes in the cell. It is also noteworthy that protein-protein interactions mediated by SLiMs represent potential targets for drug development.

References:
[1]Kumar et al., NAR 2022, doi.org/10.1093/nar/gkab975
[2] Mihalic et al., Nature Com. 2023, doi.org/10.1038/s41467-023-38015-5
[3] Sorgeloos et al., PNAS 2022, doi.org/10.1073/pnas.211464711
[4] Veinstein et al., bioRxiv 2024.08.08.607128; doi: https://doi.org/10.1101/2024.08.08.607128
[5] Lizcano-Perret et al., PLoS pathog. 2022, doi.org/10.1371/journal.ppat.1011042
[6] Allen et al., Nat Methods. 2007; 4(6):511–6. doi.org/10.1038/nmeth1048

11h30-12h30 – **Lionel Navarro** (Institut de biologie de l'Ecole normale supérieure (IBENS), France)

Extracellular small RNAs in host-bacteria interactions

Chair: Jérémy DUFAT & Ikram Bendraoua

Extracellular plant small RNAs (sRNAs) act as triggers of RNAi in interacting fungal and oomycetal pathogens. However, whether these extracellular RNA species direct gene silencing in plant-associated bacteria, which are thought to lack a canonical eukaryotic-like RNAi machinery, remains unknown. Here, I will present recent findings from the lab demonstrating the occurrence of a cross-kingdom RNA silencing phenomenon implicating the trafficking of active sRNAs from plant cells towards bacterial cells. In particular, I will report on the different extracellular sRNA species that are causal for this gene regulatory process. Finally, I will touch upon approaches that are currently developed to translate these discoveries towards novel solutions to control bacterial infections in plants and mammals.

12h30-14h00: Lunch with speakers – hall IBMP : Buffet

Afternoon November, 18 at Auditorium CRBS

Joint Immunology symposium – IMCBio (optional)

Faculté des **sciences de la vie**
Université de Strasbourg

Organized by Sylvie Fournel

14h00- **Sandrine Henri** (Centre d'Etudes des Pathologies Respiratoires, Tours, France)

Myeloid cells in health and diseases

15h00- **Eloi Verrier** (Institut de Recherche sur les Maladies Virales et Hépatiques, Strasbourg, France)

The complex interplay between hepatitis D virus and the innate immune response

16h00-16h30: Coffee break

16h30- **Matthieu Giraud** (Center for Research in Transplantation and Translational Immunology [CR2TI], Nantes, France)

Center for Research in Transplantation and Translational Immunology

18h00: Cocktail with the M2 and PhD students

Tuesday 19/11 – Auditorium CRBS

Joint Immunology symposium – IMCBio

Faculté des sciences de la vie
Université de Strasbourg

Organized by Sylvie Fournel

8h30- **Suzie Houssier** (Institut de Génétique Humaine. Laboratoire de Virologie Moléculaire, CNRS, Montpellier, France)

Crosstalk between chromatin structure and innate immune sensing

9h30– **Manuele Rebsamen** (Department of Immunobiology, University of Lausanne, Switzerland)

New players in endosomal nucleic acid sensing and autoimmunity

10h30-11h00: Coffee break

11h00- **Celine Charvet** (IGBMC, CNRS UMR 7104, INSERM U1258, Strasbourg, France)

Role of Ikaros in the pro-inflammatory signature of CD4 T cells

Wednesday 20/11 – Auditorium IGBMC

Structural Biology

8h45-9h45 - **Ohad Medalia** (University of Zurich, Switzerland)

Nuclear Lamins: Thin Filaments with Large Impact

Chair: Inzhu Tanoz & Mohsen Keshavarz-Najafi

Lamins are nuclear intermediate filaments (IFs) of metazoan cells. They assemble into fibrous structures that are positioned between the inner nuclear membrane and the peripheral chromatin, although a small fraction of lamins is present in the nucleoplasm. Lamins are required to maintain nuclear structure and, together with many interaction partners, are involved in most nuclear activities. Mutations in lamins cause a group of >14 distinct diseases called laminopathies, it is not clear how lamins are organized in vivo and how these mutations affect lamin functions. Understanding how lamins are assembled, and how mutations in lamins and lamin binding proteins affect lamin filament assembly and cellular localization is essential for understanding the their modus operandi. Moreover, lamins are intimately interacting with chromatin.

Employing cryo-electron microscopy and tomography, we studied the organization of lamins in the nuclear enevelope. We than identified the specific motif of lamin tail domain that interacts with nucleosomes. This study lay molecular basis for direct lamin-chromatrin interactions and chromatin compaction at the nuclear envelope. Our findings provide insights into the dynamic and specific interplay between lamin isoforms and chromatin, shaping chromatin architecture and epigenetic regulation

9h45-10h45 – **Andrey Kajava** (Centre de Recherche en Biologie Cellulaire de Montpellier, France)

Breaking the amyloidogenicity code

Chair: Inzhu Tanoz & Justine Decouvelaere

A broad range of human diseases including Alzheimer's disease and the transmissible prion diseases are linked to the formation of insoluble, fibrous, protein aggregates called amyloid fibrils. Numerous studies have shown that the ability to form amyloid fibrils is an inherent property of the polypeptide chain. This has led to the development of a number of computational approaches to predict amyloidogenicity by amino acid sequences. Benchmarks of these programs show that there is still a lot of room for their improvement. Recently, revolutionary progress has been made in understanding the 3D structural arrangement of amyloid fibrils due to the application of solid-state NMR and cryo-electron microscopy in combination with molecular modelling. The newly obtained structural knowledge about the amyloid fibrils opens an avenue for development of the innovative computational methods. In this talk, I will review the

existing bioinformatics methods for prediction of aggregation-prone regions in proteins and describe our efforts to improve them. As whole genome sequencing becomes cheaper, our new methods will provide opportunity to create individual risk profiles for the neurodegenerative, age-related and other amyloidoses ushering in an era of personalized medicine.

10h45-12h00: Coffee break – Cafeteria IGBMC

12h00-13h00 – **Peter Tompa** (Vrije Universiteit Brussel, Belgium)

Repeat-driven biomolecular condensation in health and disease

Chair: Inzhu Tanoz & Mohsen Keshavarz-Najafi

Structural disorder is prevalent in the proteome of eukaryotes, as intrinsically disordered proteins (IDPs) fulfil important signaling and regulatory functions[1]. Functionally, IDPs can serve as entropic chains, when their function stems directly from structural disorder, or they engage in molecular interactions, when they undergo induced folding in the presence of the partner. IDP sequences are often of low sequence complexity, i.e., they are highly repetitive, or contain a limited set of amino acids[2]. A special function of IDPs stems from that they can undergo biomolecular condensation (liquid-liquid phase separation), which is the prominent mechanism of forming membraneless organelles (MLOs), such as the nucleolus and stress granule, in the cell[3]. As such MLOs play key roles in cell signaling, the misregulation of their formation and dissolution often leads to diseases termed "condensatopathies", such as cancer and neurodegeneration. In my presentation, I will elaborate on the relationship of structural disorder, low complexity, and condensation, addressing a very special case of these relations, when pathological mutations generate highly repetitive "polymer-like" proteins – dipeptide repeats – that cause disease, amyotrophic lateral sclerosis (ALS)[4]. I will show that the underlying molecular mechanisms derive from interactions with cellular proteins driving normal condensation, severely dysregulating physiological processes in the cell[5]. I conclude my talk showing that these mechanisms give rise to novel ways of drug development, by targeting dipeptide repeats by non-conventional, polymeric molecules, such as polystyrene sulfonate[6].

References:
[1] Van der Lee, R. et al. Chem. Rev. 2014, 114: 6589–6631
[2] Tompa, P. BioEssays 2003, 25: 847-855
[3] Brangwynne, C. P., Tompa, P., Pappu, R. V. Nature Physics. 2015, 11: 899–904
[4] Boeynaems, S. et al. Mol. Cell 2017, 5: 1044–1055
[5] Van Nerom, M. et al. PNAS 2024, in press
[6] Bratek-Skicki, A. et al. bioRxiv 2023, https://doi.org/10.1101/2023.05.19.541518

13h00-14h00: Lunch with speaker – Salle Ping-Pong : Buffet

Thursday 21/11 - Auditorium IGBMC

Development

08h45-09h45 - **Timothy Saunders** (University of Warwick, center of biomechanical cell biology, United Kingdom)

The intricate interplay between mechanical interactions and signalling networks during development

Chair: Louise Bucher & Pauline Chavel

During organ development, cells undergo significant morphological and positional changes. Yet, by the end of organogenesis, the internal structure of organs is typically robustly defined. It remains an open question as to how the three-dimensional (3D) internal structure of an organ emerges reliably, particularly when there are multiple cell types interacting. Here, we utilised quantitative live imaging of the developing zebrafish myotome to unravel how muscle organisation emerges over the first 18 hours of its development. In particular, I focus on two packing challenges faced by the emerging muscle.

In the first part, I will address how preadaxial cells of the zebrafish tailbud can regulate the confluent packing of the adaxial layer of the presomitic mesoderm, the future slow muscles. We show that confluent packing can be achieved through distinct biophysical mechanisms. The specific mechanism for cell packing is determined by Hedgehog signalling, which modifies preadaxial cell movement and consequently impacts cell movement into the presomitic mesoderm adaxial layer. In wild type, confluent packing is achieved by adhesion at cell-cell contacts. Under Hedgehog signalling inhibition, confluent packing is achieved by higher cell density joining the adaxial layer through increased tissue flow. Thus, we reveal new understanding of the interaction of the genetic state and biophysical properties of cells in establishing tissue architecture.

In the second part, I will focus on how muscles maintain integrity while undergo significant shape change into elongated, force-generating fibres. We combined {\it in vivo} data with 3D morphological measures; {\it e.g.,} twisting and skew. A subset of cells are observed to undergo an ordered chiral twist, the direction and magnitude of which depends on their position within the myotome. Further, cells skew and rearrange, seemingly to facilitate close packing of neighbouring muscle fibres. We find that in mutants that disrupt cell fate or cell fusion that the packing is altered, even though the final muscle segment remained largely confluent. Motivated by experiments with restricted myotome size, we propose that boundary constraints are critical in shaping both the internal muscle structure and the overall myotome morphology.

Cellular adaptability to varying boundary constraints may be a general mechanism for ensuring robust organ morphogenesis.

09h45-10h45 – **Wolfgang Driever** (Institute of Biology [Developmental Biology], University of Freiburg, Germany)

Dynamic regulation of stem and progenitor cells in zebrafish larval neural proliferation zones

Chair: Pauline Chavel & Louise Bucher

The vertebrate nervous system develops in different phases: At neural plate stage and early neural tube, neurectodermal stem cells proliferate but do not differentiate. In embryonic stages, the brain grows from proliferation zones, where proliferation prevails, while neurons are born in adjacent areas of neurogenesis. Finally, in late embryonic and adult stages, neural stem cell niches maintain the potential to add neurons and glia. At all three stages, Delta/Notch Signalling and a family of Hairy-Enhancer of Split (HES/Her) transcription factors are central regulators of both stem cell maintenance and progression of neurogenesis. In this presentation, I would like to discuss different states of the Delta/Notch – HES/Her pathway that differentially control stem cell maintenance versus dynamics of neural progenitor neurogenesis.

We use the zebrafish larval brain between 2-5 days post fertilization as model system, because of robust neural proliferation and ease of observing neural proliferation zones in vivo. Both neural proliferation zones and neurogenic niches are organized into regions with mostly long-term neural stem cells, or predominantly progenitors. Delta/Notch signaling is a major regulator of neural stem cell (NSC) maintenance and also controls neural progenitor cell (NPC) progression towards differentiation. Typically, downstream of Notch-signalling, HES/her transcriptional repressors repress differentiation to maintain neural stem cells, and control neurogenesis progression in progenitors. However, in zebrafish, we characterized next to Notch-signaling dependent Her genes (ND: Her4/12/15/2) also Notch-independent (NI: Her6/9) Her genes. Extensive genetic analysis surprisingly revealed that predominantly NI Her genes are responsible for maintaining neural stem cells, but not ND Her genes (Sigloch et al., 2023).

To follow the dynamic expression of NI Her6 and ND Her4, we used *her6:*Her6-mNeonGreen and *her4:*Her4-mNeonGreen transgenic lines. Light sheet fluorescence microscopy revealed that Her6 expression oscillates in NSCs, while Her4 expression oscillates in NSCs and NPCs. We analyzed in detail dynamic parameters of these oscillations. Our observations are consistent with NI Her6 predominantly contributing to maintenance of NSCs, while ND Her4 may control dynamics and plasticity of progenitor cells.

We next aimed to determine differences in the transcriptomes of NSCs expressing ND or NI Her genes, and whether downstream transcriptional networks differ for both Her families. Single cell (sc) and bulk RNA-Seq analyses of Her6-mNeonGreen+ cells validated that NI *her6* is primarily expressed in both non-cycling and proliferating NSCs. Further, time series transcriptome analyses upon heat-shock

overexpression of *her6*, *her4* or activated Notch (NICD) revealed that NI Her6 activity and Notch signaling converge on shared downstream transcriptional targets. We analyzed a specific subset of NI and/or ND Her positive NSCs/NPCs from published SC RNAseq data of zebrafish brain cells (Raj et al., 2020). We identified NSC/NPC clusters differentially expressing Her genes: ND Her genes are prominently expressed in late NPCs, in which NI *her* expression is reduced. Pseudotime analysis reflecting neurogenesis stages revealed co-expression of ND *her* genes with Delta and proneural genes in NPCs, potentially contributing to a plastic progenitor state.

We further analyzed scRNAseq of ND Her vs. NI Her NSCs/NPCs, and time series transcriptome data following bursts of Notch signalling activity, Her4 or Her6 overexpression, and identify differentially active transcriptional networks in NI Her expressing NSCs versus ND Her expressing NPCs. Together, these data illustrate how in the developing brain distinct regulatory networks differentially control long-term quiescent versus active neural stem cells, and the rate of progenitor progression in neurogenesis.

10h45-12h00: Coffee break – Cafeteria IGBMC

12h00-13h00 – **Shinji Takada** (National institute for basic biology [NIBB], Japan)

Wnt-mediated community effect in maintaining stem cell populations

Chair: Marie Diebolt & Tom Lanchec

Wnt ligands play roles in many aspects of embryogenesis and homeostasis. While Wnt ligands have been shown to act over long distances from their source cells, evidence also suggests that they may act locally as autocrine and paracrine signal. However, it is not yet fully understood how their actions are spatiotemporally regulated. Therefore, we have extensively investigated the distribution and dynamics of Wnt proteins in embryos using a combination of molecular genetics, immunohistochemistry, live imaging, quantitative analysis, and protein chemistry. In this lecture, I first present some examples of our studies that have investigated the spatial distribution and dynamics of Wnt proteins in embryonic tissues. These studies suggest that most Wnt proteins are not freely diffusible in the extracellular space, but probably associated with cell surface and/or extracellular matrix proteins. Then, we addressed the question to what extent autocrine and paracrine activities contribute to Wnt function in the context of embryonic development. To this end, we focused on Wnt3a function in epiblast/tailbud cells of mouse embryos, including the neuromesodermal progenitor (NMP) cells, which are bipotent progenitor/stem cells for neural and paraxial mesoderm. Since epiblast/tailbud cells produce and receive Wnt ligands, it is possible that both autocrine and paracrine functions of Wnt3a are involved in the maintenance of NMP cells. To separate the autocrine activity of Wnt3a from its paracrine activity, we replaced endogenous Wnt3a with a receptor-

fused form that can activate signaling in producing cells, but not in neighboring cells. Mutant mouse embryos showed a unique phenotype in which maintenance of many NMP cells was impaired, although some cells persisted for long periods. The epiblast cell population of these embryos exhibited increased heterogeneity between neighboring cells in Wnt signaling level as embryogenesis progressed and were sensitive to retinoic acid, an endogenous antagonist of NMP maintenance. Thus, mutual and local paracrine function of Wnt ligands in the epiblast cell population reduces heterogeneity and achieves robustness to environmental stress. I would like to propose the importance of the local action of secreted signal proteins in embryonic development.

Reference:

- Hatakeyama Y., Saito N., Mii Y., Takada R., Shinozuka T., Takemoto T., Naoki H., Takada S. (2023) **Nat. Commun. 14**:1924. doi: 10.1038/s41467-023-37350-x.

13h00-14h00: Lunch break – Salle ping-pong : Buffet

Friday 22/11

Amphi Rothé (EOST)

Morning : Development, Genetics & Molecular Biology

09h00-10h00 – **Douglas Vernimmen** (Roslin Institute, University of Edinburgh, United Kingdom)

Deciphering the molecular function of epigenetic regulator JMJD6

Chair: John-Vincent Beauvais, Armina Jero & Zoé Rebelle

Epigenetic regulators play crucial roles in development and cellular responses to environmental stimuli, with mutations in these regulators often implicated in cancer. JMJD6, a member of the JmjC-domain containing family, exhibits a β-barrel structure with iron and oxoglutarate binding sites, and functions as an epigenetic regulator. Its lysine hydroxylase activity is well-established, yet its arginine demethylase activity remains controversial due to inconsistent findings across studies. To address the molecular function of this epigenetic regulator, we used a broad approach by the generation of JMJD6 knockout (KO) in various cell types, including haematopoietic cell lines (THP-1, K562, NOMO-1) and adherent cell lines (HEK, Kelly, HeLa). We have investigated the impact of JMJD6 deletion on growth rates, cell morphology, histone modifications and transcriptomic changes by RNA sequencing (RNAseq). The genomic occupancy of JMJD6 was also addressed by chromatin immunoprecipitation followed by high-throughput sequencing (ChIPseq).

10h00-10h30: Coffee break – Hall EOST

10h30-11h30 – **Alexis Maizel** (Heidelberg University, Centre for Organismal Studies, Germany)

Plant morphogenesis at cell scale: Getting to know where you are and what your neighbours do

Chair: Pauline Chavel & Louise Bucher

Research in my lab focuses on understanding the fundamental mechanisms of plant development, particularly root development in Arabidopsis thaliana. The main emphasis is on lateral root formation, a prime example of post-embryonic organogenesis that enhances plants' ability to forage for nutrients and stabilize their anchoring.
Our work integrates studies of hormone signaling (especially auxin), mechanical forces, cellular dynamics, and gene regulation. Our current and future research focuses on three main questions:
1. How do abutting lateral root founder cells coordinately polarize and swell?
2. How the direction of organ axes is set during post-embryonic growth?
3. How do cell growth and division contribute to the emergence of cell identities?
In my talk I will present recent results related to these points.

11h30-12h30 – **Jean-Michel Gibert** (Institut de biologie Paris-Seine, France)

Genetic bases of Drosophila dorsocentral bristle developmental plasticity

Chair: Tom Lanchec & Marie Diebolt

How developing organisms resist or respond to environmental changes is a fundamental issue. In *Drosophila*, the number of dorsocentral (DC) bristles is usually robust. Four DC bristles are observed in most individuals. We found that low developmental temperature and methotrexate, a medical drug found as pollutant in waste waters, induce ectopic DC bristles. The development of DC bristles is well known, which allows to analyse how their development is affected by these environmental factors. The proneural genes *achaete-scute* (*ac-sc*) play an essential role in the establishment of the bristle pattern. *ac-sc* expression is regulated by redundant mechanisms. *ac-sc* basal expression is controlled by a cocktail of repressive factors. In addition, modular enhancers activate *ac-sc* expression in groups of cells, called proneural clusters, among which bristle precursors will be selected by lateral inhibition, a process involving Notch signalling and auto-activation of *ac-sc*. Expression of *ac-sc* in the dorsocentral region is controlled by the DC enhancer. Interestingly, the deletion of the DC enhancer prevents the induction of ectopic DC bristles by methotrexate but does not stop low temperature to induce DC bristles. We show that methotrexate increases the activity of the dorsocentral enhancer of *ac-sc* and shows a strong synergy with mutants of factors regulating this enhancer. By contrast, temperature affects the regulation of *ac-sc* basal expression by repressive factors such as Hairy. Thus, methotrexate and cold both induce ectopic DC bristles via *ac-sc* genes but by distinct regulatory processes.

12h30-14h00: Lunch with speaker – Hall EOST : Buffet

Friday 22/11

Amphi Rothé (EOST)

Afternoon : Private sector & Industries

14h00-14h30 – **Fabienne Mathon** (SATT Conectus, France)

From Lab to Innovation : Tech Transfer Essentials

Chair: Gwenaëlle Graulier

14h00-15h30 – **Delphine Allouche & Javier Rol-Moreno** (RNA Sciences - Research & Biomarkers Department, Sanofi R&D, France)

Drug substance characterization for next generation mRNA vaccines.

Chair: Justine Decouvelaere & Bruno Del Carpio Martinez

In 2021, Sanofi inaugurated a new center to develop mRNA vaccines. The mRNA Center of Excellence (mRNA CoE) is located in two countries: France and the USA. The objective of this center is to accelerate mRNA technology for both vaccines and therapeutics. For that, more than 600 employes are working in an end-to-end platform from R&D to manufacturing. Two innovation projects are focused on the determination of RNA secondary structure at different mRNA vaccine stages and mRNA detection on active translation complexes to measure translation efficacy.

Messenger RNA (mRNA) vaccines contain *in vitro* transcribed 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). During the formulation process, mRNA is in solution in different buffers whose composition varies. These conditions can influence the mRNA structure. Secondary structure consists in the combination of different patterns such as helix, loops that are interconnected by junctions. We decided to use the SHAPE (Selective 2'-OH acylation analyzed by primer extension) method to understand and characterize structural variations of a mRNA that can be induced during the process of formulation.

Furthermore, mRNA sequence optimization is key to enhancing protein expression, which is critical in therapeutic and biotechnological applications. This study explores optimized mRNA designs by analyzing translation efficiency using polysome profiling and ribosome profiling (Ribo-Seq). Polysome profiling provides insights into mRNA translation status by quantifying ribosome-bound mRNAs, while Ribo-Seq reveals ribosome positions on transcripts, offering high-resolution data on translation dynamics. Together, these techniques help identify sequence modifications that enhance translational efficiency and define the strategies for designing novel mRNAs with improved protein yield and stability.

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