labex who am I?

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LABEX ANNUAL MEETING 2021

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Center Parcs Les Bois Francs
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ORGANIZED BY FIRST SPEAKER'S NAME

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WISCI: WHO AM I SINGLE-CELL INITIATIVE

by Caroline ANGLERAUX, IHPST & Pierre-Antoine DEFOSSEZ, EDC

- talk -

Single-cell approaches have revolutionized biology; they allow researchers to address old and new questions regarding cellular and organismal identity with enhanced resolution and in an unbiased manner.

Three factors limit the ability of Labex teams to perform Single-Cell genomics experiments:
-access to the necessary infrastructure
-cost of reagents
-bioinformatic analysis capabilities

Our plan is to lift these 3 limitations. This project will draw on existing “Université de Paris” platforms and reinforce them, it will synergize with the existing organoid projects, it will create a community of users across institutes, and it will directly benefit a large number of Labex teams.

In addition, we will promote interactions between biologists, philosophers, and historians of science around the concept of the cell and the emergence of organs and organisms.

ROLE OF LRRFIP2 IN HYPOXIA REGULATION DURING HEART DEVELOPMENT

by Laura BEN DRISS, team Maire-Sotiropoulos, IC

- poster -

Oxygen is crucial for appropriate embryonic and fetal development, including cardiogenesis. The heart is the first organ formed in the embryo and is required to provide oxygen and nutrients to all cells in the body. Embryonic cardiogenesis is a complex process finely regulated and prone to congenital malformations. It takes place in a hypoxic environment that activates the HIF-1a signaling pathway which mediates cellular and systemic adaptations to low oxygen levels.

Since inhibition or overactivation of the HIF-1a signaling pathway in the myocardium lead to severe cardiac malformations and embryonic lethality, it is important that the cellular response to hypoxia be precisely regulated. While many gene regulatory networks involved in embryonic cardiogenesis have been characterized in detail, the modulation of the response of cardiomyocytes (CM) to hypoxia has remained less studied.

We identified LRRFIP2 as a new negative cofactor of HIF-1a. Indeed, we have shown that the absence of *Lrrfip2* expression in a mouse KI model led to an enhance of many HIF-1a target genes including *Igfbp3*, *Bnip3* and *Ndufa4l2* in embryonic CM during development. As results, the absence of *Lrrfip2* led to the inhibition of the PI3K/Akt survival pathway, growth defects, mitochondrial dysfunction and to a precocious maturation of the embryonic CMs. Altogether, these defects led to the formation of a smaller heart unable to provide sufficient oxygen to the embryo and finally to a severe hypoxia and a precocious lethality.

ROLE OF LYSINE METHYLATION IN CYTOKINESIS AND CANCER

by Jérémy BERTHELET & Souhila MEDJKANE, team Weitzman, EDC

- talk & poster -

Our laboratory studies how methyltransferases control cell fate and how their deregulation can alter cell identity and contribute to cancer development.

We began the project looking at a methyltransferase enzyme that adds epigenetic marks to histone tails to regulate gene expression. Observing that this enzyme is also in the cytoplasm, we questioned whether it might be involved in other cellular processes. This led us to the unexpected finding that lysine methylation also regulates cytokinesis, the final steps in the separation of cells at the end of mitosis. Deregulation of this mechanism might lead to the disrupted cell division and polyploidy that are drivers of cancer.

This project addresses the question of how aberrant lysine methylation alters the cytokinetic process leading to unfaithful cytokinesis, polyploidy and cancer promotion.

SMALL NUCLEOLAR RNAS IN NORMAL AND PATHOLOGICAL MUSCLE DIFFERENTIATION

by Baptiste BOGARD, team Francastel, EDC
- talk & poster -

RNA splicing is a critical step for gene expression in eukaryotes, any disruption lead to human diseases like the Myotonic Dystrophy type 1 (DM1). During splicing, introns are removed from the precursor mRNA (pre-mRNA) and are quickly degraded, although a subset escapes degradation and provides precursors of small regulatory non-coding RNAs (sncRNA), including small nucleolar RNAs (snoRNAs) that guide RNA modifications and are at the center of posttranscriptional regulation. In addition, introns can be retained in the mature RNA; this alternative splicing is a developmental switch that can affect the production of sncRNAs.

We set out to document AS introns in the context of human muscle cells and address its functionality during normal differentiation and in cells from patients with DM1. We developed a medium RNAseq dedicated to the systematic identification of new sncRNAs in muscle cells and identified a hundred new snoRNAs that will enrich annotation of the human genome. In order to validate them as genuine snoRNAs, I assessed their presence in ribonucleoprotein complexes and checked their accumulation in the nucleolus. Furthermore, I uncovered a family of snoRNAs (SNORA40) whose expression levels increased during normal muscle differentiation but were drastically reduced in DM1 cells. Functional studies showed that reduced levels of SNORA40 family impaired cell fusion in normal cells whereas rescued levels of SNORA40 partially restored cell fusion in DM1 cells.

In conclusion, my work sheds novel light on the regulation of muscle differentiation by providing new actors and mechanisms to consider as well as unconventional biomarkers for splicing diseases.

DEVELOPING IPSC-DERIVED CORTICAL AND SPINAL ORGANOIDS TO DECIPHER THE MOLECULAR AND CELLULAR DEFECTS UNDERPINNING NEURODEVELOPMENTAL DISEASES

by Kamal BOUHALI, enSCORE platform, IJM/EDC

- poster -

Alterations of neurodevelopment lead to a wide spectrum of disease manifestations. These include intellectual disabilities, impaired learning and adaptive function, impaired sensory as well as motor deficits, some of which are associated with CNS structural defects, such as micro- or macro-cephalies or opened neural tube.
The enSCORE plateform has been created to support an interdisciplinary consortium of teams with complementary skills and knowledge that aim to address the high complexity of these neurodevelopment mechanisms and their intricated interplays using induced pluripotent stem cells (iPSCs) derived from healthy donors or patients. Using appropriate and standardized cortical and spinal organoid differentiation protocols, these teams aim to study the interplays between mechanical and transcriptional cues underpinning normal and pathological neurodevelopment.
Over the first 9 months, the enSCORE plateform has set up standardised human iPSC culture, banking and Quality Controls (8 distinct lines validated so far), cortical neural organoids differentiation (with help from the Mezger team) and their immuno-fluorescence and FACS-based phenotyping. We trained three members of the Doye lab, and recently generated our first genome-edited iPSC lines bearing a homozygous mutation causing autosomal recessive microcephaly and Seckel syndrome spectrum disorders.
We aim for the following year to help Labex teams to generate other genome-edited mutant iPSC lines, set up spinal organoïd differentiation (with V. Ribes team), help a postdoc from the Passaro lab to elaborate neural organoïds recapitulating the cellular interactions between the developing vascular and nervous systems, and refine the phenotyping of organoids, notably regarding epigenetics, transcriptomics, and mechanical cues.

THE EPISTEMIC AND ONTOLOGICAL VALUE OF STOCHASTICITY IN CELLS STRESS RESPONSE. THE CASE OF ALTERNATIVE START-CODON SELECTION

by Marco CASALI, team Huneman, IHPST
- poster -

Chance in gene expression is typically addressed as “noise” in the context of transcription. By contrast, since translation is usually seen as “a linear conversion with one predictable, unambiguous outcome” (Putois et al 2015), its relation with stochasticity is seldom addressed. I have already explored the role of chance in transcription (Casali and Merlin 2020) arguing that it can be more than just noise, i.e. chance is an epistemic element having a specific role in biological explanations. This project represents an opportunity to extend this analysis to translation. Since transcription and translation processes happen in similar physico-chemical conditions, there is no a priori reason, theoretically speaking, that stochasticity would not affect this other stage of gene expression. This finds empirical confirmation in recent experimental research that clearly shows the pervasive influence of stochasticity on translation (e.g., Boersma et al 2019). Furthermore, in situations of cellular stress, translation privileges the synthesis of alternative proteins rather than standard ones (Sriram et al 2018). This event, called alternative start-codon selection, has recently been qualified as stochastic (Boersma et al 2019). This project aims to provide a philosophically sound, empirically-based theoretical framework to unravel the meaning and role of stochasticity in translation. I want to analyze the role of chance in alternative start-codon selection, to clarify the epistemic and ontological value of chance in this specific research context. Alternative proteins synthesized by chance are often seen as “error[s] in translation” (Boersma et al 2019, p. 459) or “translational noise” (Pauli et al 2015, p. 6). By contrast, I will ask whether the production of alternative proteins could be considered as a sort of bet-hedging strategy enacted by cells to face environmental stresses. If, following Wu Orr et al (2019), these proteins can have a role in tumor prevention and the maintenance of cellular homeostasis, then it seems they are essential for cell integrity. If so, we should re-examine intracellular processes at their origin from this perspective, and in particular, re-evaluate the possible explanatory role of their stochastic character.

ROLE OF MECHANICS AND CELL-CELL ADHESION IN THE EMERGENCE OF COLLECTIVE CELL MIGRATION

by Victor CELLERIN, team Ladoux/Mège, IJM
- poster -

Mechanical plasticity in epithelial cells has a pivotal role in cancer, wound healing, and morphogenesis. We are questioning how force transmission and biomechanical signaling at cell-cell contacts can support multi-scale polarization in collectively migrating cell ensembles, change of cell identities, and different migration modes. To do so, we set-up and characterize a minimal and original experimental model, consisting n epithelial cell trains confined on one dimensional track.

We used a bottom up approach, based on force measurement experiments, we showed that single cells exert an asymmetric dipolar force pattern during their migration. Moving to collective migration using cell doublets we add a level of complexity, since with the maintenance of biochemical and physical interaction at cell-cell contacts, symmetry breaking can now arise both at the scale of the doublets and/or of individual cells within the collective.

We first observed a switch in the migration modes where the cell doublets are redistributing their forces from the junction to the substrate when the cells are more elongated. By tuning cell-cell adhesion we demonstrated that destabilizing the cell-cell junction increase the redistribution of the forces to the substrate. We demonstrate that cell elongation correlates with force redistribution to the substrate for longer train (up to 15 cells) while collectivity increases the proportion of forces transmitted through cell-cell junctions. By quantifying adhesion complexes at cell-substrate and cell-cell interfaces, we provide mechanisms that lead to emergent modes of collective migration.

ELUCIDATING THE PROXIMAL ORIGIN(S) OF SARS-COV-2

by Virginie COURTIER-ORGOGOZO, team Courtier, IJM

- poster -

Discussions are ongoing about the identity of SARS-CoV-2, the virus responsible for COVID-19 pandemic. Is it fully natural, artificial, intentional or accidental? This project aims to use available sequences of coronaviruses to try to answer this question.

ANALYSIS OF TRANSCRIPTIONAL NETWORKS AND CELLULAR COMMUNICATIONS DURING SKELETAL MUSCLE HYPERTROPHY

by Léa DELIVRY, team Maire-Sotiropoulos, IC
- poster -

Skeletal muscle is mainly composed of myofibres, multinucleated cells, and other cell types such as stem cells, macrophages, FAPs, etc. It is an extremely plastic tissue capable of changing over time: it can atrophy, hypertrophy, and regenerate. During hypertrophy, there is an increase in fiber volume and an activation of the muscle stem cells, which then proliferate and fuse to the growing myofibre. It has been shown that macrophages and FAPs help the regeneration process but nothing has been studied at the level of hypertrophy.

Our aim is to understand how the myofibers (myonuclei) respond to the muscle skeletal hypertrophy and to investigate the role and the communications of the various cellular types present in the skeletal muscle during hypertrophy. To answer these questions, we performed a single nucleus RNA-seq on 10-week-old female mice. The model of hypertrophy that we used was compensatory hypertrophy. Two conditions were performed: one with plantaris muscles from sham-operated mice and one with plantaris muscles from mice subjected to surgery inducing hypertrophic growth for 7 days. This technique allows us to obtain the RNA-pre-messenger of all nuclei present in the muscle. Thanks to bioinformatics analyses, we were able to carry out UMAP. We found two new type of myonuclei : MISO and MIOV.

NEURAL ORGANOIDS TO INVESTIGATE INTERPLAYS BETWEEN MECHANICAL AND TRANSCRIPTIONAL CUES UNDERPINNING NORMAL AND PATHOLOGICAL NEURODEVELOPMENT

by Valérie DOYE, IJM, Valérie MEZGER, EDC & Vanessa RIBES, IJM

- talk -

Based on the unprecedented opportunity offered by pluripotent stem cells-derived organoids and the combination of our diverse and complementary expertise, we aim at investigating cross-talks between mechanical and transcriptional cues that dictate the spatiotemporal dynamics in cell fate and shape, underpinning the normal or pathological development of the human central nervous system.

PARAMECIUM POLYCOMB REPRESSIVE COMPLEX 2 PHYSICALLY INTERACTS WITH THE SMALL RNA BINDING PIWI PROTEIN TO REPRESS TRANSPOSABLE ELEMENTS

by Sandra DUHARCOURT, team Duharcourt, IJM
- talk & poster -

The epigenetic landscape is established and maintained by different protein complexes in transcriptionally active versus silent regions of the genome. Polycomb Repressive Complex (PRC2) deposits the histone mark H3K27me3 on silent protein-coding genes, while transposable elements are repressed by DNA and/or H3K9 methylation. Yet, there is increasing evidence that PRC2 also targets and even silences transposable elements in representatives of several distantly related eukaryotic lineages, including the ciliate Paramecium. What determines the specificity of recruitment for PRC2 to the TEs that it represses is unknown.

Here, we demonstrate that Paramecium PRC2 undergoes a direct physical interaction with the RNA interference machinery and we precisely delineate the mechanistic basis of PRC2 recruitment to TEs and its molecular participants. Our findings not only shed light on small RNA-guided PRC2 targeting to TEs, but also reveal parallels with TE recruitment pathways for other classes of repressive histone methyltransferases and therefore provide a new evolutionary context for understanding TE silencing more broadly.

INTERPLAY BETWEEN THE ONCOPROTEIN FAK AND PYK2 AND THE MOLECULAR SCAFFOLDS BETA-ARRESTINS

by Hervé ENSLEN, team Marullo, IC

- poster -

FAK (Focal Adhesion Kinase) and Pyk2 (Proline-rich kinase-2) are related non-receptor tyrosine kinases. FAK is ubiquitous while Pyk2 expression is restricted. Their mode of regulation is different and their functional outputs may be similar or distinct depending on the cell context. The expression and activation of FAK and Pyk2 are increased in various cancers, and correlate with tumor progression and poor prognosis. FAK and Pyk2 inhibitors are in phase I/II clinical trials for several types of cancer. It is therefore very important to better understand their regulation. FAK and Pyk2 are activated downstream of integrins, G Protein-Coupled Receptors (GPCRs) and Receptor Tyrosine Kinases (RTKs) in response to both mechanical or chemical changes occurring in the cell environment. They are both kinases and protein scaffolds. In inactive or active form, they both interact with common or distinct signaling protein partners, in the cytoplasm, at the plasma membrane (receptors, focal adhesions) and in the nucleus, to regulate cell adhesion, migration, proliferation and survival. For example, FAK is enriched at Focal Adhesions (FA), intracellular molecular complexes where integrins connect the Extracellular matrix (ECM) to the actin cytoskeleton, regulating their turnover with FAK being known to act there both as a direct mechanosensor and downstream effector. Pyk2 may also be localized to FA depending on cell type and displays similar effects to FAK.

ß-arrestins (ß-arr1 and ß-arr2) were initially identified because they promote the desensitization and internalization of G protein-coupled receptors (GPCRs). They are also protein scaffolding platforms that bind numerous signaling proteins (some of which are shared with FAK and Pyk2), dynamically regulating their localization and/or activities in the cytoplasm, at the membrane and in the nucleus downstream of different types of receptors (integrins, GPCRs and Receptor Tyrosine Kinases, (RTK)), or even via receptor-independent mechanisms. Through these interactions ß-arrs regulate cell adhesion, migration, proliferation and survival. ß-arr deregulation is correlated with tumor progression. We recently identified ß-arrs as new regulating partners of FAK. FAK regulation involves a dual role of ß-arrs, which inhibit FAK in the cytoplasm of resting cells while driving its activation at the plasma membrane by GPCR-stimulated G-proteins (Alexander et al, Cell Mol Life Sciences, 2020). We have now demonstrated that ß-arrs also regulate Pyk2 but depending on cell activation status, the regulation is similar or different from those reported for FAK. This may be due to a distinct Pyk2/ß-arr mode of interaction we have documented. We have now generated molecular and genetic tools to investigate the consequences of the interaction of ß-arrs with FAK and Pyk2 on their nuclear localization and function in the nucleus. Our results not only provide new important information on the regulation of FAK and Pyk2 but also how they may contribute to an understanding of physiopathological processes during cancer where misregulation of ß-arrs, FAK and Pyk2 is established.

MEMBRANE MECHANISMS THAT BLOCK POLYSPERMY

by Sophie FAVIER, team Vaiman, IC
- talk & poster -

Fertilization is the biological process by which a sperm and an egg fuse to produce a zygote that develops and becomes a new being. In case more than one sperm fuse with an egg in an abnormal process called polyspermy, the zygote is not able to develop to full term. For polyspermy to occur, several sperm must pass through the zona pellucida (ZP), a glycoprotein envelope that surrounds the oocyte, and then fuse with the membrane of the oocyte. Our data showed that during *in vitro* fertilization in mice, in 70% of cases, a single sperm crosses the ZP of the oocyte and fuses. In the remaining 30%, more than one sperm crosses the ZP, yet in most cases only one of them fuses with the oocyte. The absence of fusion of the other sperm that have passed through the ZP therefore reveals the existence of a blocking mechanism in polyspermy. Our aim is to elucidate this mechanism.

Nowadays, 2 oocyte membrane proteins have been identified as essential for fusion: Juno, an adhesion protein whose Izumo1 receptor is found on the head of the sperm, and CD9, a tetraspanin with a role of membrane organizer. However, recent data indicate that these molecules are released from the oocyte membrane on vesicles, in small quantities before a fusion occurs, then in very high quantities after fusion. Our hypothesis is that this release has therefore by itself the potential to deprive the gametes of the possibility to properly interact for fusion and thus to block polyspermy By combining molecular biology, quantitative imaging, micromanipulation and laser ablation, I studied the impact of the JUNO protein on the fusion capacity of the spermatozoon by two complementary approaches, one using the spermatozoa directly recovered in the EPV of zygotes, and the other sperm artificially placed in the presence of JUNO. At the same time, in order to kinetically characterize the release of JUNO and its attachment to spermatozoa entering the EPV, I undertook to genetically modify JUNO expressed by the oocyte, so as to make it fluorescent without altering its function.

SEGREGATION OF LONG-LASTING DNA DAMAGE DURING CELL DIVISION AND ITS POTENTIAL IMPACT ON STEM CELL FATE

by Juliette FERRAND, team Polo, EDC
- talk -

The faithful segregation of intact genetic material and the perpetuation of chromatin states through mitotic cell divisions are pivotal for maintaining cell functions and identities across cell generations. Checkpoint and repair mechanisms prevent the transmission of damaged chromatin to daughter cells. Still, most exogenous mutagens generate long-lasting damage, which can be segregated during mitosis. How this segregation is controlled is unknown, and the potential contribution of mitotic chromatin marks to such control mechanism is an attractive but still unexplored possibility.

Our study reveals an unexpected connection between damaged chromatin marking and asymmetric segregation of DNA lesions during mitotic cell divisions. The potential relevance of asymmetric damage inheritance for cell identity maintenance has not yet been investigated. To tackle this question, we are currently taking advantage of asymmetric stem cell divisions in human intestinal organoids, which generate daughter cells of different fates. By combining 2D organoid cultures, DNA damage induction and stem cell division tracking by microscopy, we examine whether DNA damage is inherited non-randomly during asymmetric stem cell divisions. Because stem cells are the gatekeepers of tissue homeostasis, we hypothesize that the preferential transmission of the damaged genetic material to the differentiated cells may safeguard the stem cell compartment from the deleterious effects of DNA damage accumulation. Investigating the response to DNA damage in physiological models such as organoids in culture will improve our understanding of the maintenance of tissue homeostasis upon genotoxic stress conditions.

EVOLUTION OF STEM CELLS AND REGENERATION IN ANIMALS

by Eve GAZAVE, team Vervoort, IJM

- poster -

The “Stem cells, Development and Evolution” team at the Institut Jacques Monod studies the evolution of stem cells and regeneration in animals.

The main biological model studied by the team is the annelid worm, *Platynereis dumerilii*, an emerging model species for developmental and evolutionary studies. We characterize, with a large variety of molecular, cellular, and genomic tools, the amazing regenerative abilities of this animal, with the general aim to determine, through comparative analyses, whether regeneration in animals relies on conserved mechanisms and genetic programs or not. We are, in particular, developping cell lineage and single-cell transcriptomics approaches to characterize cell identities and cell identity changes during regeneration. We also study the importance of epigenetic regulations in this process and aim to identify the cellular mechanisms and the signaling pathways that control regeneration. In addition, using a combination of experimental and *in silico* approaches, we also aim to understand how stem cells involved in development, growth and regeneration have evolved in animals, and help to better understand the concept of stem cell itself. We notably have started to define and compare the molecular signatures of a large variety of stem cells from different animals using published single-cell transcriptomic data.

3D SHAPE OF EPITHELIAL CELLS IN A CURVED ENVIRONMENT

by Sylvie HENON, team Physique du Vivant, MSC

- talk & poster -

Epithelia are ubiquitous tissues with a wide variety of functions and shapes, ranging from flat to highly curved, for instance in intestinal villi or pulmonary alveoli. Different morphogenetic events, such as gastrulation or branching morphogenesis, are correlated with changes in the curvature of epithelia. The development of a physical framework to account for cell shape in epithelia is therefore an important challenge to understand various normal and pathological biological processes, such as epithelial morphogenesis or cancer metastasis.

We propose an experimental procedure to validate a minimal physical model for epithelial cell shape in 3D. We cultured Madin-Darby canine kidney (MDCK)epithelial cells on substrates with a sinusoidal profile, which allowed us to measure cell shape on different positive and negative curvatures. We also performed a quantitative analysis of the correlation between cell thickness and curvature during ventral furrow formation in the early Drosophila embryo. We found that in both the early Drosophila ventral furrow and MDCK epithelia, cells are thicker when positively curved (in the valleys of the sinusoidal substrates) than when negatively curved (on the ridges). The influence of curvature on epithelial cell shape could not be understood with a model using only surface energies. However, the addition of an apical line tension was sufficient to quantitatively account for the experimental measurements. Our work opens the way to a quantitative analysis of the coupling between curvature and cell identity.

DECODERS - DECODING LOCUS-SPECIFIC MOLECULAR NETWORKS USING BIOTIN-BASED PROXIMITY LABELING

by Guillaume JANNOT, team Palancade, IJM
- poster -

For several decades, various approaches have been extensively used to illuminate the identity of protein-protein interaction (PPI) in pre-assembled biological complexes. Proximity-dependent BIOtin IDentification (BioID) recently emerged as a powerful strategy for proteomic analysis of macromolecular complexes. In BioID, a protein of interest (POI) is fused to a promiscuous biotin ligase or peroxidase and expressed *in vivo*. The fusion enables biotinylation of proteins interacting in proximity to the POI in a dynamic and spatio-temporal manner. De novo biotinylated complexes are then isolated and characterized by mass spectrometry or NGS analyses. Importantly, BioID can be adapted to decipher the molecular interactions that take place at discrete genomic loci, by targeting the biotinylating enzyme to specific sequences.

Therefore, in order to decrypt the molecular composition of chromatin landscapes in several distinct situations, the WHOAMI Labex-funded DECODERS platform seeks to implement a general workflow to spatially resolve proteomic and transcriptomic interactions at individual loci. Four main objectives are planned over the next three years: i) creation of the platform; ii) implementation of the DECODERS workflow in pilot experimental systems; iii) adaptation of the DECODERS workflow to alternative experimental models; iv) large-scale decoding of locus-specific molecular interaction networks. This bottom-up approach will pave the way for the characterization of the molecular and mechanistic basis of identity, a central question for the Labex WHOAMI.

PLATFORM "BIOMECHANICS OF THE CELL"

by Mireille LAMBERT, team Lambert, IC
- poster -

The mission of the platform, BioMecan’IC (Institut Cochin, Paris), is to optimize and develop technical approaches permitting the measurement and study of the transmission and integration of forces at the sub-cellular, cellular, and tissue levels. BioMecan'IC provides training on the use of equipment and technologies, so that teams can be independent for the development of the proposed biophysical approaches. BioMecan’IC also collaborates on innovative projects and ensures constant technological monitoring.

MALE GENITAL LOBE MORPHOLOGY AFFECTS THE CHANCE TO COPULATE IN DROSOPHILA PACHEA

by Michael LANG, team Courtier, IJM
- talk -

Left-right asymmetries have evolved mutiple times independently in animal genitalia, but the constraints that maintain such a specific left-right identity in a species population are unknown. *Drosophila pachea* males have asymmetric genitalia with a pair of external lobes where the left lobe is about 1.5 times longer than the right lobe. We show that a longer left lobe confers an advantage to engage into copulation with a female over a male with a shorter left lobe. This selective advantage may be mediated through courtship signals or by facilitating the establishment of genital contacts. It contributes to the preservation of male left-right asymmetry in *D. pachea*.

DECIPHERING THE ROLE OF FOXO1 IN DEFINING THE IDENTITY OF EFFECTOR CD4 T CELLS

by Théo LEVEL, team Lucas, IC
- poster -

The transcription factors of the forkhead box O family (Foxo1, Foxo3, and Foxo4) are critical in T cells at the crossroad of different processes, such as quiescence, cell survival and T-cell trafficking. More recently, another layer of complexity was added to this picture by the discovery that Foxo transcription factors may also control the differentiation of naive CD4 T cells into effector helper T cells. Indeed, a specific conditional knockout of Foxo1 in mouse T cells (Foxo1TKO mice) impairs the generation and the suppressive functions of induced regulatory T cells. Moreover, we have recently demonstrated that Foxo1 is a T-cell intrinsic inhibitor of the Type 17 helper T-cell (TH17) program (Laine, A. et al. J Immunol 195, 1791-1803. 2015). Surprisingly, the involvement of Foxo1 in types 1 and 2 helper T-cell (TH1/TH2) generation has not yet been studied with precision. We have recently observed that higher percentages of effector CD4 T cells from the secondary lymphoid organs of Foxo1TKO mice are able to produce IFN-γ (TH1) and IL-13 (TH2) *ex vivo* and that Foxo1-deficient naive CD4 T cells are prone to differentiate into TH1 and TH2 cells *in vitro*.

In light of our preliminary results, the goal of this project is to decipher the role of the transcription factor Foxo1 in the differentiation of naive CD4 T cells into effector TH cells. We hypothesize that Foxo1 adjusts the response of naive CD4 T cells to their cytokine environment by determining the identity of the effector T cells arising from their activation.

MOLECULAR BASES AND BIOLOGICAL FUNCTIONS OF CENTRIOLE ROTATIONAL ASYMMETRY IN HUMAN CELLS

by Paula MARTIN GIL, team Azimzadeh, IJM
- talk -

Centrioles are very conserved microtubule-based structures that form the core of the centrosome, the main microtubule-organizing center in most animal cells. Centrioles exhibit a highly conserved nine-fold symmetry, which results from the organization of the triplets of microtubules that compose them. In unicellular eukaryotes, centrioles carry asymmetrically attached appendages required for controlling centriole position within the cell. The assembly of these appendages involves molecular asymmetries between the microtubule triplets of the centriole.

We have discovered that molecular asymmetry also exists within human centrioles despite their lack of visible structural asymmetry. The project aims at understanding the molecular bases of centriole rotational asymmetry and elucidating its roles in animal cells

THE NON-CANONICAL ROLES OF DNA METHYLATION DURING EPIGENETIC REPROGRAMMING

by Ana MONTEAGUDO SANCHEZ, team Greenberg, IJM

- poster -

During early mammalian development, most of the parental epigenetic information is erased. Subsequently, de novo DNA methylation occurs via action of the DNMT3 enzymes. In parallel, other regulatory transitions that accompany early cellular commitments occur.

In the Greenberg lab, we use an embryonic stem cell differentiation system that recapitulates this period to determine how de novo methylation interacts with other epigenetics marks. For example, we are trying to understand the relationship between the polycomb repressive complex and DNA methylation. So far, we have found several loci that lose H3K27me3 during the epigenetic reprogramming and coincidentally gain DNA methylation in our system. Additionally, we want to discover if the *de novo* methylation has an impact on genome conformation; to do so we have profiled the CTCF binding profile at different points of the epigenetic reprogramming process and we have observed that the gain of methylation is associated with the loss of CTCF binding at certain loci.

DECODERS: DECODING LOCUS-SPECIFIC MOLECULAR NETWORKS

by Benoit PALANCADE, team Palancade, IJM
- talk -

Cellular identity relies on the maintenance, the expression and the transmission of genetic and epigenetic information. These processes are achieved by the orchestrated action of a myriad of cis-acting genetic elements and DNA-bound trans-acting factors, including chromatin regulators. While the molecular composition of chromatin is extremely heterogeneous depending on the genetic locus and the cellular status, methodological limitations have precluded so far the characterization of the full repertoire of factors acting at given genomic positions.

The aim of this transversal project is to decode such locus-specific molecular interaction networks, by implementing proximity ligation-based proteomics and transcriptomics at individual genomic sequences. With the representative diversity of genome functions, physiological situations and experimental models studied by Labex members, this bottom-up approach will pave the way for the characterization of the molecular and mechanistic bases of identity, a central question for WHOAMI.

MOLECULAR DISSECTION OF THE MECHANISMS REGULATING CELL DIVISION IN SPACE AND TIME DURING DEVELOPMENT

by Lionel PINTARD, team PINTARD, IJM
- poster -

Multicellular organisms contain a large number of cells (3x10^13 in humans) and a wide variety of cell types with a defined identity. Except for the gametes that are produced through a specialized cell division program (meiosis), all these cells are generated through mitotic cell divisions. Beginning with the reunification of the parental genomes at the beginning of life, mitosis ensures the transmission of the genetic material between the two daughter cells. This process is thus essential for the transmission of identity between generations.

The “Cell cycle and Development” team investigates several aspects of meiotic and mitotic cell divisions using the nematode C. elegans, which provides a rich context to dissect the mechanisms regulating cell division during development. We also apply emerging paradigms to other systems, including human cells. We employ a variety of approaches, including genetics, cell biology, biochemistry, biophysics, and proteomics. Here, we present the scientific axes of the laboratory and the main results obtained thanks to the Labex “WHO AM I”.

1/ How is mitotic entry triggered and regulated in a developmental context (asynchronous cell divisions)?

2/ How are the male and female pronuclear envelopes remodeled during the first mitosis to permit the reunification of the parental genomes at the beginning of life?

3/ How does the microtubule-severing enzyme Katanin sculpt microtubules to permit the assembly of the female meiotic spindle?

T LYMPHOCYTE ASYMMETRY UPON CHEMOKINE STIMULATION: INTERPLAY BETWEEN CAMP AND CYTOSKELETON

by Clotilde RANDRIAMAMPITA & Morgane SIMAO, team Randriamampita, IC

- poster -

Cell migration is a central phenomenon in the life of an organism, both during development and physiological responses or in pathological configurations. In all cases, whatever the cells, migration is preceded by the establishment of cell polarization making it possible to acquire an asymmetry thus defining an anteroposterior axis and therefore the direction of migration. Many proteins especially from cytoskeleton network are involved in the establishment of this polarization as well as in directionality control.

Mainly by using dynamic cell imaging, we aim to understand the signaling pathways controlling these processes in T lymphocytes whose stimulation by chemokines triggers their polarization and their migration. We have identified cAMP as a key factor able, through protein kinase A activation, to regulate both T cell polarization and directionality. Indeed, during cell migration, local bursts in cAMP at the cell front are able to induce a rapid relocalization of cortical actin thus promoting direction changes(1). More recently, we have focused on early chemokine stimulation steps and tried to determine whether T lymphocytes subjected to a uniform stimulation, will develop a polarization axis randomly or whether some intrinsic cellular element will favor a specific direction. In this context, we have identified the centrosome, actin cytoskeleton and protein kinase A as key players.

(1) Simao, M. et al., cAMP bursts control T cell directionality by actin cytoskeleton remodeling. Frontiers in cell and developemntal biology (2021). doi.org/10.3389/fcell.2021.633099

MUSCLE INDUCED DIFFERENTIATION: INTERPLAY BETWEEN MECHANICAL AND ELECTRICAL EFFECTS (MIDMEE)

by Myriam REFFAY, team Biofluidique, MSC
- talk -

During development, tissues experience forces and electrical stimulations that influence their biological functions. However, the initiation of various metabolic, transcriptional or mechanical responses to environmental stimuli remains largely unexplored, pointing out the need to look at interplay between electrical coupling, mechanical stimuli and transcription factors. Muscle cells are at the very heart of this debate. Multi-scale organization and generated forces are the hallmarks of the functional skeletal muscle tissue. It thus represents a reference model to disentangle effects of electrical and mechanical stimuli at the scale of a single cell but also in the production of collective behaviour. Precursor myoblasts reveal as being influenced by physical properties (through adhesion and force generation or electrophysiological stimulation) both in term of proliferation, migration, nuclear positioning. Dissecting these mechanisms in the context of the muscle cell differentiation enhancement appears as a challenge for muscle tissue engineering.

MIDMEE project aims at determining correlation between stimulation and spatially-resolved differentiation in an innovative program. It proposes to unravel the role of mechanical stress and membrane resting potential in the appearance, maintenance and regeneration of muscle system. The complementarity of the different teams involved in this collaboration will allow to work from cellular to tissue scale, and correlate physics and biology tools. First, micro-patterning appears as an appealing candidate to monitor cell environment through the 2D constraints of their adhesion. We will correlate various clues of cell differentiation starting from transcriptomics to electrical conduction. In a second part of the project, 3D assembled micro-tissue obtained by magnetically manipulated cells labeled with superparamagnetic nanoparticles will be stimulated through micro-magnet stretcher and will allow exploring these two factors in 3D.

MYELIN ON-CHIP: STUDYING NEURO-GLIA INTERACTION IN THE PNS

by Hugo SALMON, team Biofluidique, MSC
- poster -

Peripheral neuropathies affect about 5% of the general population after the age of 50 and are caused by a wide set of factors among whom diabetes, genetic causes, toxic (such as chemotherapies, pollutants), and life habits. The oxidative stress on neurons and Schwann Cells (SCs) plays a central role in them and the treatments usually lead to poor functional recovery. Extracellular vesicles (EVs) seem to play an important role in regulating this stress and more generally in the myelination mechanisms. They could constitute a potential avenue for new biotherapeutics for the peripheral neuropathies.

In this context, I develop a myelinating model of the peripheral nervous system from mice embryonic cells. Taking advantage of compartmentalized microfluidic architectures, I plan to monitor the myelination as well as demyelination/remyelination induced after oxidative stress. I am adapting an analytical workflow to monitor the EVs collected in the chip and monitor them during the development, maintenance, degeneration, and regeneration of myelin. The myelin on-chip will also constitute *in vitro* neuropharmacological assays to study the regenerative and anti-oxidation potency of biotherapeutics based on EVs.

THE LYSINE METHYLTRANSFERASE SETDB1 REGULATES MITOSIS THROUGH INCENP (INNER CENTROMERE PROTEIN) METHYLATION

by Gilles TELLIER, team AIT-SI-ALI, EDC
- poster -

SETDB1 is a lysine methyltransferase (KMT) belonging to the SUV39 subfamily which methylates the lysine 9 of the histone H3 (H3K9). SETDB1 catalyzes all forms of H3K9 methylation but mainly di- and tri-methylation, promoting transcriptional repression. This process affects cancer progression through epigenetic silencing of tumor suppressor genes and modulation of signaling pathways controlling cell proliferation, migration and invasion. All the members of the SUV39 family are dysregulated in cancer. In particular, SETDB1 overexpression is a common feature in different cancers, such as lung, breast, melanoma, liver and colon cancers, favoring tumorigenesis.
Beyond histones, all the members of the SUV39 family methylate many non-histone substrates, including UBF, p53, DNMT1, HP1 and p63, which are involved in carcinogenesis. For example, SETDB1 participates in liver cancer cell growth via p53 methylation, which increases p53 stability. Moreover, SETDB1 methylates the kinase Akt promoting its kinase activity and oncogenic functions.
Our project aimed to exhaustively identify the non-histone substrates of SETDB1 using proteomic approaches. Among SETDB1 partners and/or substrates, we identified a significant set of proteins involved in mitosis and macroscale chromosome organization regulators, including the Chromosome Passenger Complex (CPC). The loss of CPC function results in lagging chromosomes, segregation errors and cytokinesis failures. This promotes genomic instability, which contributes to oncogenesis by altering the balance of critical growth and death pathways, along with the overall expression of oncogenes. Our data indicate a potential role of SETDB1 in the regulation of the cell fate at the non-chromatin level, via the methylation of the CPC, in addition to H3K9 methylation at the chromatin level. My project aims at deciphering the role of the CPC subunit INCENP methylation by SEDTB1. We show that SETDB1 could have an unexpected role in mitotic progression regulation via methylation of non-histone substrates, suggesting that SETDB1 can impact cell proliferation independently of its role in gene expression regulation through H3K9 methylation.

IN VITRO RECONSTITUTION OF THE CROSSTALK BETWEEN ACTIN AND VIMENTIN FILAMENTS

by Duc Quang TRAN, team Leduc, IJM
- talk & poster -

The cytoskeleton, mainly composed of three types of interconnected biopolymers: actin, intermediate filaments and microtubules, is a complex biopolymer network, which is responsible for most of the mechanical properties of the cell including the maintenance of its integrity. Among them, actin forms dynamic networks which can quickly assemble and disassemble in response to the extracellular environment, but single actin filaments are not mechanically resistant to deformation. On the contrary, vimentin intermediate filaments form long-lived networks with slow assembly/disassembly kinetics, and possess remarkable mechanical properties such as highly stretchable, hyper-elastic and resistance to large deformations. Although actin and vimentin have different structural and physical properties, there is growing evidence that they work in coordination to control cellular functions such as cell migration, control of cell shape and mechano-sensing. However, it remains unclear how these two networks physically interact with each other.

In this project, we want to investigate how actin and vimentin networks regulate each other's properties, either directly or through specific passive and active crosslinkers, and how the crosstalk between them is modulated under external stresses. By using simplified systems reconstituted in vitro with purified constituents, we expect to unravel novel molecular mechanisms involved in mechano-sensing in networks of actin/vimentin, which will be subjected either to internal stress through molecular motors, or to external stress using a unique stretching device.

MUSCLE INDUCED DIFFERENTIATION: INTERPLAY BETWEEN MECHANICAL AND ELECTRICAL EFFECTS (MIDMEE)

by Lorijn VAN DER SPEK, team Biofluidique, MSC

- poster -

During development, tissues experience forces and electrical stimulations that influence their biological functions. However, the initiation of various metabolic, transcriptional or mechanical responses to environmental stimuli remains largely unexplored, pointing out the need to look at interplay between electrical coupling, mechanical stimuli and transcription factors. Muscle cells are at the very heart of this debate. Multi-scale organization and generated forces are the hallmarks of the functional skeletal muscle tissue. It thus represents a reference model to disentangle effects of electrical and mechanical stimuli at the scale of a single cell but also in the production of collective behaviour. Precursor myoblasts reveal as being influenced by physical properties (through adhesion and force generation or electrophysiological stimulation) both in term of proliferation, migration, nuclear positioning. Dissecting these mechanisms in the context of the muscle cell differentiation enhancement appears as a challenge for muscle tissue engineering.

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SPATIO-TEMPORAL COORDINATION BETWEEN LAMINA DEPOLYMERIZATION AND MITOTIC SPINDLE ELONGATION PROMOTES THE UNIFICATION OF THE PARENTAL GENOMES IN THE C. ELEGANS ZYGOTE

by Griselda VELEZ-AGUILERA, team PINTARD, IJM

- poster -

A new life begins with the unification of the maternal and paternal genomes. In the C. elegans zygote, after fertilization, parental genomes are physically separated into two pronuclei, each surrounded by a nuclear envelope, and meet for the first time during mitosis. A scission event that removes membranes on the juxtaposed oocyte and sperm pronuclear envelopes is required to allow the parental chromosomes to mingle on the metaphase plate.

Here, we demonstrate that the mitotic Polo-like kinase PLK-1 phosphorylates the lamin (LMN-1) to promote lamina depolymerization, pronuclear envelopes scission, and parental genomes unification. Expression of non-phosphorylatable lamin (LMN-1 8A), which affects lamina depolymerization during mitosis, is sufficient to prevent the pronuclear envelopes scission and therefore, the mixing of the parental chromosomes into a single nucleus in daughter cells. Besides PLK-1-mediated lamina depolymerization, we also show that astral microtubule pulling forces contribute to pronuclear envelopes scission, by facilitating the removal of the lamina and by promoting mitotic spindle elongation. Our observations suggest that temporal coordination between lamina depolymerization and mitotic spindle elongation induces pronuclear envelopes scission, and in turn, facilitates the reunification of the parental chromosomes after segregation.

INTEGRITY OF THE SHORT ARM OF NUCLEAR PORE Y-COMPLEX IS REQUIRED FOR MOUSE EMBRYONIC STEM CELL GROWTH AND DIFFERENTIATION

by Annalisa VERRICO, team Doye, IJM
- poster -

The precise control and coordination of cell division and differentiation are essential in development; if altered, changes in cell number and/or identity can arise, and ultimately lead to numerous pathological conditions. The components of nuclear pore complexes (NPCs) are critical players in development and pathologies. However, studying contributions of individual NPC subunits to these processes in vertebrates has long been impeded by NPC complexity and the lack of efficient genetic tools.

Here, we use genome editing in mouse embryonic stem cells (mESCs) coupled to quantitative image analysis to characterize the role of NPC structural components, focusing on the short arm of the Y-complex that comprises Nup85, Seh1 and Nup43. We show that Seh1 and Nup43, although dispensable in pluripotent mESCs, are required for their normal cell growth rates, their viability upon differentiation, and for the maintenance of proper NPC density. mESCs with an N-terminally truncated Nup85 mutation (in which interaction with Seh1 is greatly impaired) feature a similar reduction of NPC density. However, their proliferation and differentiation are unaltered, indicating that it is the integrity of the Y-complex, rather than the number of NPCs, that is critical to ensure these processes.

EX VIVO BIOMIMETIC INTESTINAL MODEL FOR DEVELOPMENTAL STUDIES

by Wang XI, team Ladoux-Mège, IJM

- talk -

Intestinal epithelium (IE) constitutes the second largest epithelium in human body and exposes to a highly dynamic and complex microenvironment. Healthy intestine withstands various mechanical stimuli, extreme pH variations, contains different biochemical/physical gradients and hosts trillions of bacteria. The single-layer epithelium organizes into highly regular tridimensional (3D) crypt and villus structures, where cells of varied functions are found at distinct areas and undergo rapid proliferation, differentiation, coordinated cell migration, and extrusion. These spatiotemporal organization and dynamics preserve the intestinal stem cell niche and enable constant tissue replenishment, making IE a unique dual functional barrier for both uptakes of nutrient and protection against pathogens. Recent research has revealed that the villus-crypt architecture and environmental factors play critical roles in IE homeostasis and mechanisms in physiological and pathological conditions. However, current planar petri-dish-based and organoid culture systems lacks the control of different environmental parameters.

Combining micro-engineering techniques and cell biology, we address such a disadvantage by developing hydrogel scaffolds that represent similar 3D crypt-villus architecture of intestinal epithelia. These scaffolds are further functionalized with proteins and integrated into microfluidic or Transwell set-ups for mimicking *in vivo* biochemical gradients. We present that primary intestinal stem cells from organoid culture can grow on our 2D and 3D scaffolds and replicate key features of the small intestine. Our novel approach to an *ex vivo* intestinal model can serve as a versatile platform for broad bio-mimicking applications of IE and is adaptable to model other tissues. For example, it would be applicable to decipher mechanisms for the development of IE layer and its homeostasis and to study the pathogenesis of rare congenital intestinal diseases, which are technically difficult to investigate *in vivo*.

ELUCIDATING THE MOLECULAR MECHANISMS OF TRANSCRIPTION TERMINATION BY THE RNA POLYMERASE III

by Juanjuan XIE, team Porrua/Libri, IJM
- talk & poster -

Transcription needs to be terminated precisely and efficiently to avoid the interference between neighbor genes. Most eukaryotic organisms possess three different RNA polymerases (RNAP). Among them, RNAPIII transcribes of tRNA genes and other short non-coding genes. Unlike RNAPII, that strictly requires extrinsic factors to terminate transcription, the current model posits that RNAPIII termination relies solely on a particular DNA sequence, which are typically stretches of thymines (Ts) that are presumably sufficient for both RNAPIII pausing and its release from the DNA.

In this study, we unveil an interaction between RNAPIII and a well-characterized transcription termination factor for RNAPII-dependent non-coding genes, the helicase Sen1. We combine genome-wide high-resolution mapping of transcribing RNAPIII with highly-purified in vitro transcription termination assays to analyse the interplay between nucleic acid elements and the trans-acting factor Sen1 in termination of RNAPIII transcription. We find that an important fraction of RNAPIIIs normally read through the primary terminator (i.e. the first T-tract downstream of the 3' end of genes) and terminate at downstream secondary terminators. Importantly, preventing the interaction of Sen1 with RNAPIII decreases the efficiency of termination, indicating a global role for Sen1 in promoting RNAPIII termination. We show that T-tracts are only partially efficient in promoting the release of paused RNAPIII from the DNA and that Sen1 can stimulate RNAPIII release by translocating along the nascent transcript. We provide evidence that Sen1 functions mainly at regions with secondary terminators. Conversely, we find that hairpin-like structures that typically form at RNAPIII transcripts near the primary terminator can also stimulate RNAPIII release.

Taken together, our results redefine the rules that govern efficient termination of RNAPIII transcription.