LABEX WHO AM I – Closing conference 2024

Exploring identity: from molecules to individuals

Posters Abstracts

BIOLOGY

#1 Parasite protein secretion takes the reins of bovine gene expression

Jérémy BERTHELET, Epigénétique & Destin Cellulaire

Pathogens co-evolve with their hosts, developing sophisticated strategies to bypass defenses and manipulate physiology. For example, Theileria parasites can transform and immortalize bovine leukocytes, inducing cancerous phenotypes. The lack of a parasitophorous vacuole membrane in Theileria-transformed cells, suggests unique mechanisms to hijack host signaling pathways. Although some secreted parasite proteins, such as TaPin1, have been identified, our understanding of these mechanisms remains limited. Our lab has begun an ambitious project focused on mapping and characterizing the entire set of proteins secreted by the parasite (the secretome), investigating how they rewire the host epigenome, and ultimately determining their role in sustaining cancer phenotypes. A pilot study based on this project has identified two parasitic proteins capable of translocating into the host cell nucleus. One of them, SPORF1, exhibits a highly specific punctate localization. Proteomic analysis of its interactors revealed a potential connection with two major gene regulating complexes: CtBP and non-canonical Polycomb Repressive Complex (ncPRC1 1.3). Relocalization of these complexes by SPORF1 could impact the local expression of certain genes, in particular by altering H2K119Ub profiles, for which the PRC1 complex is responsible. We are currently investigating the molecular impact of SPORF1 on these complexes and, more broadly, its role in the significant restructuring of bovine gene expression during infection. This study could provide the first evidence for a direct mechanism by which Theileria parasites can modify the epigenome of its host cell via unprecedented hijacking of the host PRC1 complex.

#2 enSCORE platform: Engineering organoids since 2021

Kamal BOUHALI, Epigénétique & Destin Cellulaire

The enSCORE platform was established in 2021 to support an interdisciplinary consortium addressing complex questions in developmental neurobiology. It focuses on using organoid models derived from induced pluripotent stem cells (iPSCs), which remain the only existing models of human prenatal brain development that allow for the integration of genetic and pharmacological approaches.
Funded by the Labex Université Paris Cité “Who am I?” under the transversal project "Neural organoids to study the interactions between mechanical and transcriptional signals underlying normal and pathological neurodevelopment", enSCORE uses iPSCs from both healthy donors and patients with neurodevelopmental disorders to build in vitro model for neurodevelopment studies.
The enSCORE platform has three main missions:

- Optimization, standardization, and characterization of human iPSC cultures: enSCORE provides support and training in human iPSC culture, ensuring quality control.
- Design and generation of transgenic lines through genome editing: The platform works with researchers to model pathologies by replicating patient mutations via CRISPR/Cas genome editing in iPSC lines.
- Production and characterization of neural organoids (cerebral and spinal): enSCORE employs a variety of protocols to generate cerebral and spinal organoids. These organoids are phenotyped using cryostat sectioning, immunofluorescence labeling, and flow cytometry.

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#3 Identification and characterization of new substrates of the lysine methiltransferases SMYD2 and SMYD3 involved in tumor progression

Camille BRECHOTEAU, Epigénétique & Destin Cellulaire

Methylation of histone proteins is an epigenetic mark that plays a role in the regulation of many genes. This function has long been studied, but it appears that many non-histone nuclear and cytoplasmic proteins are also methylated. Although first identified as histone lysine methyltransferases, SMYD2 and SMYD3 are two enzymes that can also methylate non-histone substrates. Interestingly, SMYD2 and SMYD3 act as a tumor facilitator in a wide range of cancers. Little is known about the molecular mechanisms that explain their contribution to the tumor process.
We aim to identify and characterise new substrates of these enzymes to explain their involvement in tumour progression. Many enzyme-substrate interactions are transient and labile, limiting their detection by conventional immunoprecipitation techniques. This is why we use APEX-BioID (Ascorbate Peroxidase Biotin Identification) technology. In the presence of BP and H2O2, the APEX enzyme is able to biotinylate proteins within a radius of 10 nm. The biotinylated proteins can be identified by mass spectrometry after streptavidine pull-down. Three cell lines, MHCC97H (hepatocellular carcinoma), A549 (non-small cell lung cancer) and HeLa (cervical cancer) were selected as relevant models for our study because their oncogenic phenotypes are associated with SMYDs protein levels. In HeLa cells, SMYD2 and SMYD3 are known to affect cytokinesis, and their overexpression leads to nuclear and genomic defects that may play a role in tumorigenesis. Three constructs were stably integrated into

each cell line using lentivectors: SMYD2-APEX, SMYD3-APEX and APEX (control). The APEX-BioID system was optimised for all models. Preliminary mass spectrometry data validate known substrates for SMYD2. The next step will be to select five targets for SMYD2 and SMYD3 based on the mass spectrometry results. We will test whether these targets are methylated by our enzymes and find out on which lysine residues this reaction takes place. One substrate for each enzyme will be used for the following study. New cell models with non-methylable substrates, loss and gain of function of SMYD2 or SMYD3 will be generated. These cell lines will allow us to study the cellular and molecular processes associated with these methylation events. Overall, this project will help to understand how methylation contributes to cancer.

#4 The PIWI-interacting protein Gtsf1 controls the selective degradation of small RNAs in Paramecium

Olivia CHARMANT, Institut Jacques Monod

Ciliates undergo developmentally programmed genome elimination, in which small RNAs direct the removal of transposable elements during the development of the somatic nucleus. 25-nt scnRNAs are produced from the entire germline genome and transported to the maternal somatic nucleus, where selection of scnRNAs corresponding to germline-specific sequences is thought to take place. Selected scnRNAs then guide the elimination of transposable elements in the developing somatic nucleus. How germline-specific scnRNAs are selected remains to be determined. Here, we provide important mechanistic insights into the scnRNA selection pathway by identifying a Paramecium homolog of Gtsf1 as essential for the selective degradation of scnRNAs corresponding to retained somatic sequences. Consistently, we also show that Gtsf1 is localized in the maternal somatic nucleus where it associates with the scnRNA-binding protein Ptiwi09. Furthermore, we demonstrate that the scnRNA selection process is critical for genome elimination. We propose that Gtsf1 is required for the coordinated degradation of Ptiwi09-scnRNA complexes that pair with target RNA via the ubiquitin pathway, similarly to the mechanism suggested for microRNA target-directed degradation in metazoans.

#5 Understanding the impact of biological sex and sex hormones on immunity to urinary tract infection

Léa DELTOURBE, Institut Cochin

Urinary tract infections (UTI) are extraordinarily common with a pronounced sex disparity in incidence (50% of women and 5% of men). Men are at higher risk of chronic infection while women have more recurrent UTI, suggesting that immunity to UTI is also sex-biased which could be due to sex differences in hormone levels. To improve precision medicine for both sexes we need human based studies to better understand the impact of biological sex, and particularly of sex hormones, on immunity to UTI. Therefore, I used two different approaches, a human cohort and a human organ on chip-based model. First, we assessed the concentration of major steroid hormones and their intermediates in the Milieu Intérieur (MI) Cohort, composed of 1000 healthy donors. Using integrative analyses, we observed that interindividual variability, while largely explained by sex and age, is also associated with donors' physiological characteristics, behavior, genetic factors, plasma protein concentration, and potentially with healthy aging. Second, we used previously generated datasets and the newly generated steroid hormone level dataset to analyze associations with history of UTI in the MI donors. We observed that the MI cohort represents well UTI in the general population and we identified a few associations which could contributes to sex differences in UTI. Finally, to dissect mechanisms of sex differences in host-pathogen interactions and immunity to UTI at early time points, we developed a human 3D bladder-

on-chip model which recapitulates important features of the human bladder, such as a multilayered urothelium. This model can be constructed with elements from either female or male origin such as urine, endothelial cells or monocytes and can then be infected with uropathogenic bacteria. This model constitutes a new platform to study the mechanisms of the pathophysiology of UTI with the possibility to focus on sex differences. Overall, this project highlighted many factors which impact levels of steroid hormones and several associations with UTI history in a large cohort. It also established a new and powerful tool that can be used to understand the contribution of sex on the development of UTI. My findings will provide a strong foundation for follow-up studies on how sex impacts immunity in particular in the bladder.

#6 Stability of ecologically scaffolded traits during evolutionary transitions in individuality

Guilhem DOULCIER, Macquarie University

Evolutionary transitions in individuality are events in the history of life leading to the emergence of new levels of individuality. Recent studies have described an ecological scaffolding scenario of such transitions focused on the evolutionary consequences of an externally imposed renewing meta-population structure with limited dispersal. One difficulty for such a scenario has been explaining the stability of collective-level traits when scaffolding conditions no longer apply. We show that the stability of scaffolded traits can rely on evolutionary hysteresis: even if the environment is reverted to an ancestral state, collectives do not return to ancestral phenotypes. We describe this phenomenon using a stochastic meta-population model and adaptive dynamics. Further, we show that ecological scaffolding may be limited to Goldilocks zones of the environment. We conjecture that Goldilocks zones—even if they might be rare—could act as initiators of evolutionary transitions and help to explain the near ubiquity of collective-level individuality.

#7 An in vitro model to investigate neural crest patterning and its influence on cell plasticity

Saverio FORTUNATO, Institut Cochin

During mouse embryonic development, premigratory cranial neural crest cells (CNCC) are a heterogeneous cell population that carries positional information reflective of their spatial origin along the anteroposterior axis. This positional patterning is erased during EMT as cells migrate ventrally to contribute to craniofacial structures. The significance of premigratory CNCC patterning and whether it affects future cell fate decisions remains unclear. In addition, it has been recently demonstrated that distal regulatory elements in loci associated with CNCC mesenchymal derivatives are accessible for future activation prior to migration. Whether this epigenetic priming is heterogeneous and carries positional information through the erasure of the transcriptional program remains undetermined. Through a combination of multi-omics approaches and phenotypical analyses we are aiming to investigate the transcriptional programs and epigenetic markings underlying CNCC positional identity and how these affect CNCC proliferative, migratory and differentiative capabilities. To study these phenomena, we developed an in vitro neurosphere model of the anterior neuroepithelium. Our neurospheres are produced by aggregation in 96 well plates, which make this model higly customizable and suitable for multiplexing experimental conditions. This model succesfully recapitulates CNCC induction, anteroposterior patterning and migration. Through the use of this model, generated from an Otx2-GFP; Gbx2-TdTomato mESC line, we are studying the epigenetic program of anterior and

posterior CNCC, and it will be used to test a large number of candidate genes to assess their significance to CNCC plasticity.

#8 Molecular and cellular consequences of KIF14 methylation

Charles FRISONROCHE, Epigénétique & Destin Cellulaire

Protein methylation is a cellular process that has long been studied exclusively with respect to histones. Yet, this post-translational modification is one of the most abundant in the cell, and the underlying molecular mechanisms are just beginning to be explored. We are investigating SMYD2, a lysine methyltransferase which is overexpressed in many cancers and is known to methylate numerous non-histone substrates. However, the various players involved in SMYD2's oncogenic effect are still poorly understood. We recently identified KIF14, a kinesin protein, as a novel SMYD2 substrate. KIF14, in addition to its cytoskeletal remodeling activity, is involved in membrane remodeling processes such as cytokinesis or ciliogenesis. Furthermore, KIF14, like SMYD2, is often considered to be oncogenic. We characterized KIF14 lysine 297 as monomethylated by SMYD2 using different biochemical approaches. The synthesis of an antibody specifically recognizing this modification (K297me1) enabled us to confirm that it was indeed present in the cell. This methylation takes place in the unstructured N-terminal of KIF14, a protein interacting domain known to be highly modified. We will unravel how KIF14 methylation influences the protein's interactions with its partners through a combination of microscopy, proteomic and biophysical strategies. We hope to understand how this modification affects the kinesin's cellular functions, such as its motility on microtubules and its role in cytokinesis. Ultimately, we would like to determine whether SMYD2-mediated KIF14 aberrant methylation in cancer contributes to the oncogenic process and tumor progression.

#9 NODAL signaling is required for blastocyst development in the mouse

Futura GATTOBIGIO, Institut Jacques Monod

Preimplantation development in the mouse begins at the zygote stage (embryonic day E0.5) and lasts 4 days, during which cell divisions and cell differentiation lead to the establishment of 3 cell populations - the trophectoderm, the epiblast (Epi) and the primitive endoderm (PrE) - which form a blastocyst capable of implanting in the uterus. The morphogen NODAL, a well-known TGF family member, is expressed continuously during blastocyst formation, but its role in this process is still unclear. NODAL expression begins in the inner cell mass (ICM) of the compacted morula (embryonic day E2.75), and is dynamically regulated in the Epi and PrE cells that derive from the ICM once the blastocyst is formed, up to the time of implantation. All components of the SMAD2,3-dependent ACTIVIN/NODAL signaling pathway are also present at these stages. However, to date, no requirement for NODAL has been identified before peri and post-implantation stages. The fact that ACTIVIN also is expressed in preimplantation embryos, and that both ligands are expressed by the uterus of pregnant females, raised the possibility that the absence of zygotic NODAL might be compensated at these early stages. Indeed, we found that preimplantation embryos cultured in the presence of a pharmacological inhibitor of ACTIVIN/NODAL signaling show Epi and PrE maturation delays. However, treatment of embryos with NODAL or ACTIVIN showed that these two molecules affect the growth and maturation of Epi and PrE differently. Furthermore, E2.5 Nodal -/- embryos cultured ex-utero for 48h form blastocysts that consist solely of a layer of TE surrounding a lumen with few or no inner cells. This phenotype - more severe than that obtained with the inhibitor of the pathway, or that described for SMAD2,3 double KO embryos - can be rescued by the addition of recombinant NODAL, but not by ACTIVIN. These results reveal an early role for NODAL in the development of the ICM, apparently

independent of its usual signaling pathway. I will present a detailed characterization of the ontogeny of the Nodal -/- phenotype, based in particular on the analysis of live imaging data.

#10 Epithelial density controls cell migration through a Focal Adhesion-nucleus mechanotransduction pathway

Thomas GERMIER, Institut Jacques Monod

The perception of cell density within a cohesive tissue is thought to underlie the regulation of tissue growth, homeostasis and regeneration in multicellular organisms. However, the mechanisms by which cells sense and adapt their behaviour to their density within the tissue remains largely unknown. To address this question, we used genetically encoded biosensors of protein mechanics and enzymatic activity, genetic and pharmacological perturbations and quantitative fluorescence microscopy on cultured model epithelia to study mechanotransduction pathways downstream of cell density. We found that epithelial cells respond to decreased density by increasing the size of their focal adhesions, leading to mechanical relaxation of the mechanotransducer Vinculin and release of its competitive binding with the Focal Adhesion Kinase (FAK) and the Extracellular Signal-Regulated Kinase (ERK). As a result, FAK can directly bind and activate ERK within a cytoplasmic complex. Cytoskeletal tension applied to the LINC (Linkers Of the Nucleoskeleton and Cytoskeleton) complexes then facilitates Importin-7-dependent nuclear translocation of the two kinases. In the nucleus, ERK contributes to chromatin decompaction and post-translational modifications. In addition, the low density favours translocation to the nuclear envelope of the mechanosensitive phospholipase PLA2, an ERK target that is phosphorylated in proportion to the nuclear localization of ERK. Finally, PLA2 activity promotes cell migration within the epithelium. Overall, our results support a model in which epithelial cell density controls cell migration via a pathway involving ERK and PLA2 and a combination of mechanically gated interactions and activities from adhesion complexes to the nuclear envelope.

#11 Identifying essential genes in host-parasite interactions using CRISPR technologies

Marisol GIACOMINI, Epigénétique & Destin Cellulaire

Theileria parasites are apicomplexan pathogens that infect cows and they have the unique ability to hijack host cell signaling pathways and shape phenotypes. Theileria annulata parasites infect bovine B cells or macrophages and turn them into immortalized, hyperproliferating, invasive cancer-like cells. The parasite has evolved sophisticated strategies to drive host cell phenotypes and induce genetic and epigenetic reprogramming; nevertheless, the molecular mechanisms remain unclear. This project investigates the importance of host genes in the interaction with the intracellular pathogen T. annulata. I predict that certain bovine proteins are essential for the survival of the parasite. To identify essential host genes, I will conduct a genome-wide CRISPR screen to systematically knock out host genes and determine their impact on parasite growth and persistence. I will use parasite survival markers (such as parasite specific H3K18me3 or the p104 antigen) to monitor parasite load. I will exploit these two parasite proxies using flow cytometry to sort the top, or lowest, 10% cells and then sequence CRISPR library barcodes to identify host genes essential forvpathogen persistence. Moreover, I will investigate drug treatments to identify genes whose disruption render the parasite resistant to elimination. By leveraging flow cytometry and barcode sequencing, I will identify a full set of genes required for parasite persistence in the host cytoplasm and maintenance of cancer phenotypes. I will validate these hits by conducting a secondary microscopy-based screen, investigating parasite and host morphological characteristics in Cas9-expressing cells, and I will use live

microscopy imaging to examine parasite dynamics during host cell division. Ultimately, this genome-wide analysis of essential host genes will highlight the intricate mechanisms of host-parasite interactions. I will define how these genes impact the host genome and epigenome in order to identify targets for drug development.

#12 Mechano-adaptation of cancer cells: understanding the role of VAPA-mediated contact sites

Mélina HEUZE, Institut Jacques Monod

During their lifetime, cancer cells need to adapt to a mechanically-evolving micro-environment that in turn influences their integrity, meaning their shape, survival, proliferation, adhesion, and migration, which drive tumor progression. This mechano-adaptation depends partly on the mechanosensing properties of focal adhesions that can be tuned at different levels. In this context, some lipidic messengers called phosphoinositides play a key role in the mechano-adaptation of cancer cells. We have recently identified a new regulator of cell motility, called VAPA, which modulates the homeostasis of phosphoinositides at the plasma membrane. VAPA is an ER-resident protein acting as a tether at membrane contact sites where exchange of lipids, Ca2+, and metabolites take place. Here, we show that during cell motility, ventral ER-plasma membrane contact sites anchor close to focal adhesions, at the time of focal adhesions disassembly. Our results reveal that VAPA is essential to stabilize ventral ER-PM contact sites and to maintain their spatial and temporal connection to FA. Caco2 adenocarcinoma epithelial cells depleted for VAPA exhibit disorganized actin cytoskeleton, fail to properly disassemble FA and generate lower traction forces, suggesting a role for VAPA in the mechanical integrity of cancer cells. Recently, we have developped optogenetic tools that will allow us to directly assess the role of VAPA at focal adhesions. To conclude, our results reveal unprecedented functions for VAPA-mediated membrane contact sites during cancer cell motility and mechano-adaptation and bring new insights in the understanding of cancerogenesis.

#13 Decoding Locus-Specific Molecular Interaction Networks

Mohamed MLIH, Institut Jacques Monod

Cellular identity and plasticity are underlined by loci-specific interaction networks that primarily drive genome organization, expression and stability. To gain insight into these processes, the Decoders Project aims at unravelling molecular interaction networks at specific genomic regions, using advanced proximity labelling techniques. For this purpose, we have developed a suite of biotinylation-based versatile tools that can be applied to identify protein networks involved in genomic processes such as RNA biogenesis, DNA replication, epigenetic modifications, and the response to DNA damage, across a wide range of experimental models, from yeast to human. Our experimental strategy relies on the combination of targeting moieties, specific for the loci of interest (e.g. dCas9/gRNA), and biotinylating enzymes such as TurboBioID or APEX, allowing for proximity labeling of neighboring proteins. In situations not amenable to transgenesis, an in-house purified Protein A-APEX conjugate can be used for biotinylation around a target epitope bound by a specific antibody. Once biotinylated, proteins are purified by streptavidin pulldown and potential interacting partners are analysed by mass spectrometry in collaboration with the IJM Proteomics Facility. In this context, we have used distinct procedures to assess (i) the proteome composition of a model R-loop-forming gene in yeast, (ii) the epigenetic regulators of programmed genome rearrangements in Paramecium, and (iii) dynamic changes in the chromatin landscape in response to UV damage in human cells. As exemplified with these recent applications, the project is well positioned to identify new protein interactions, thus advancing our understanding of complex biological processes.

#14 Deciphering the impact of NUP107 mutations on kidney and brain disorders using hiPSC derived models

Ona PRAT PLANAS, Institut Jacques Monod

Nuclear pore complexes (NPCs) are huge protein assemblies that mediate the essential trafficking between the cytoplasm and the nucleus. Despite their presence in all nucleated cells, some of their constituents (the nucleoporins, NUPs) have been linked to human hereditary diseases affecting specific cell types and organs. In particular, mutations in nucleoporins of the Y-complex (a key subassembly of the NPC), NUP107 amongst others, were reported to induce steroid resistant nephrotic syndrome (SRNS), a fatal kidney disease. However, the molecular mechanisms by which nucleoporin defects lead to SRNS are unknown. To tackle this question, we decided assess the impact of SRNS-causing mutations using human induced pluripotent stem cells (iPSCs) and their differentiated derivatives obtained using 3D kidney organoid differentiation, as well 2D-differentiation of iPSCs towards podocytes. We generated iPSC lines derived from an SRNS patient carrying a mutation within NUP107 and rescued this mutation by gene editing. SRNS-causing Y-NUP mutations may impair NPC assembly or lead to the formation of defective NPCs. These defects may be present in all cell types but have a more pronounced impact on cells that require more efficient transport, or on those with inherently low NPC density in normal tissues. Alternatively, NPC defects may lead to nuclear envelope instability affecting its mechanical properties in specific cell types (notably podocytes which are subjected to glomerular hydrostatic pressure). To address these issues, we have combined quantitative and super-resolution immunofluorescence using various anti-NUP antibodies. Data obtained in iPSCs revealed an alteration of nuclear basket assembly in NUP107-SRNS mutant cells, a phenotype reminiscent of those observed in mESC Y-complex mutants (Orniacki et al., 2023). Similar studies will now be performed in podocytes. Furthermore, we have initiated an analysis of the impact of this mutation on nucleocytoplasmic transport using an optogenetic nuclear transport reporter (NLS-LEXY). These analyses, along with functional studies using glomerulus-on-chips based assays (as described in Roye et al., 2021) will help us to understand how mutations in ubiquitously expressed nucleoporins lead to podocyte-specific phenotypes.

#15 A BMP mediated PAX3/7 transcriptional activity switch creates cell fates patterns in the developing spinal cord

Robin RONDON, Institut Jacques Monod

The emergence of cellular diversity in the developing central nervous system is controlled by the combined activity of secreted signals and transcription factors. The mechanisms by which neural progenitors integrate these two signals have yet to be deciphered. For this, we investigate the interplay between BMPs and the transcription factors PAX3 and PAX7 during the specification of dorsal spinal relay and associating neurons. Our data show that PAX activity prevents ventralisation of relay and associating progenitors by two distinct molecular mechanisms depending on BMPs exposure. In absence of BMPs, PAX mediated associating neurons specification relies on a repressive PAX transcriptional activity. Accordingly, PAX bind cis-regulatory genomic regions nearby key ventral specifier genes and their activity is required for the deposition of the repressive H3K27me3 mark on these regions. In presence of BMPs, this PAX repressive activity is maintained onto some ventral gene loci. In addition, at the same time, part of PAX is relocated to other cis-regulatory regions, some of which are nearby relay neurons specifier genes and are opened and activated by the PAX. This transactivating activity of the PAX is necessary for relay neurons generation. In addition, we have showed using chick embryos that PAX mediated activation or repression on specific loci will create

patterns of expression in the neural tube. Altogether, our data demonstrate that BMP signalling acts as a switch for PAX transcriptional activity, increasing their specification potential tenfold.

#16 Role of Autophagy in Intestinal Stem Cells (ISCs) division

Alessia RUBIOLA, Institut Cochin

Intestinal stem cells (ISC) undergo rapid proliferation, renewing the entire intestinal epithelium every 4 days. They are crucial for maintaining the homeostasis of the epithelium and are also the origin of colorectal cancer. It is therefore essential to understand the precise mechanisms that regulate their proliferation and differentiation. Our published data indicate that intestinal functions are maintained in autophagy-deficient animals, despite increased p53- dependent apoptosis in Lgr5+ ISC. Interestingly, we showed that p53 ablation in ATG7-deficient mice favors tumor growth. Our recent proteomic analysis, performed on isolated Lgr5+ ISC, indicates that loss of ATG7 impacts on the expression of critical components involved in centrosome and kinetochore-centromere composition. We derived organoids from duodenal intestinal crypts of VillinCreERT2Atg7$^{lox/lox}$, VillinCre^{ERT2}p53$^{lox/lox}$, VillinCreERT2Atg7$^{lox/lox}$ p53$^{lox/lox}$ mice treating them with 4OH-Tamoxifen ex-vivo, to assure a cell-autonomous effect. To further validate the autophagy inhibition model, we generated Atg5 KO organoids using CRISPR-Cas9 technique. We showed that the loss of Atg7 or Atg5 protein causes defect in centrosome organization, mitotic spindle architecture, and chromosome segregation. Moreover, we maintained organoid cultures for six months and conducted longitudinal genomic evolution studies, revealing genome instability following autophagy inhibition and p53 loss, which were associated with neoplastic transformation. Finally, we showed that the mitotic defects associated with autophagy deficiency can sensitive the cell to the effect of taxanes-based chemotherapy.

#17 Understanding regeneration success in animals through a comparative study: insights from the annelid Platynereis dumerilii

Zoé VELASQUILLO RAMIREZ, Institut Jacques Monod

Regeneration is a widespread process among animals but very variable in scale: from scarce cell types to the renewal of complex structures. The marine annelid Platynereis dumerilii, an emerging model to study regeneration, can restore both the posterior part of its body and its appendages named parapodia after amputation. During these processes a wound epithelium closes the wound, then a mass of undifferentiated cells, called blastema, forms and differentiates to reform the lost structures. We aim at understanding the cellular and molecular mechanisms driving the success of regeneration in our model. To characterize parapodia regeneration and the reformation of the different tissues and structures, we studied the expression of known molecular markers of appendage development and tissue differentiation. The importance of cellular proliferation was assessed through EdU incorporations to label, localize, and quantify dividing cells at different stages of regeneration. To evaluate the need for this proliferation, anti-proliferation agent hydroxyurea was used to block cell division at different stages. Furthermore, we showed that from early stages, cells in the regenerating structure express ‘stem cell genes’. Finally, we are studying the influence that certain biological parameters can have on parapodia regeneration. To identify common elements in both types of regeneration exhibited by the worms, we are using a scRNA-seq approach. By analysing and comparing the cell populations that we are currently annotating of the posterior part and the parapodia, in physiological conditions as well as at the blastema stage, we aim at understanding the composition of these structures and the cell types needed for the success of regeneration.

#18 Understanding the impact of intra-tumoral heterogeneity found in metaplastic breast cancer on tumor progression

Hélène VIGNES, Institut Jacques Monod

Cancers, particularly breast cancers, exhibit significant intra-tumoral heterogeneity, which poses considerable therapeutic challenges. Metaplastic breast cancer, despite its rarity, manifests enhanced aggressiveness and poor prognostic outcomes, largely attributable to its heterogeneous nature. It is characterized by the coexistence of distinct tumor subpopulations with both epithelial and mesenchymal cancer cells. Our hypothesis is that this spatial intra-tumoral heterogeneity favors tumor progression via a cooperation between the different subpopulations of cancer cells. To investigate this, patient-derived xenografts (PDXs) from two originally distinct patients are used to obtain primary cell cultures in the form of tumoroids. Upon culturing the tumoroids on a substrate (glass coated with fibronectin or type I collagen), the preservation of the two excepted sub-populations, epithelial and mesenchymal, as well as their spatial segregation, is observed. Our findings reveal that the epithelial subpopulation expresses E-cadherin (E-cad+) and is surrounded by mesenchymal cells negative for E-cadherin (E-cad-). Interestingly, epithelial cells exhibit invasive behavior through collective cell migration at the interface with mesenchymal cells. They infiltrate either beneath the mesenchymal cells on glass, or within a gel of type I collagen. This result suggests that the presence of mesenchymal cells is necessary for inducing invasion. A competitive dynamic exists at the interface between the two subpopulations, since at the expense of the expansion of the epithelial cells, the mesenchymal cells detach from the substrate (extrusion phenomenon) and also form 3D multilayer cell structures locally at the interface. We observed that the formation of these multilayered structures, that could promote tumor growth, is associated with an increase in secreted fibronectin by mesenchymal cells that may help to glue the cells together. We are currently investigating whether these aggregates of mesenchymal cells confine the epithelial cells inducing their invasive behavior (mechanical contribution) or whether some biochemical are also needed to guide the invasive behavior of the epithelial cells (biochemical contribution). In summary, our findings suggest that the presence of different cancer subpopulations within the same tumor may play a pivotal role in breast tumor progression.

#19 Role of the lysine methyltransferase SETDB1 in the TGF\u03b2-induced fibrosis in Duchenne Muscular Dystrophy

Maeva ZAMPERONI, Epigénétique & Destin Cellulaire

Duchenne muscular dystrophy (DMD), one of the most severe muscular dystrophies, is due to the lack of functional Dystrophin, which causes chronic injury and thus, muscle degeneration. DMD is also characterized by fibrosis, that is an abnormal deposition of extracellular matrix (ECM) resulting in the replacement of functional tissue by fibrotic connective tissue. The main pathway that sustains this excessive pro-fibrotic response is the TGFβ pathway, which is overactivated in DMD. Since the histone lysine methyltransferase SETDB1 is i) involved in muscle differentiation (Beyer et al, 2016) and, ii) interacts with the TGFβ pathway in the cancer context (Du et al, 2018), we have investigated its role in our model of DMD myotubes derived from iPSCs or immortalized myoblasts (Caputo et al, 2020). First, we have found that SETDB1 accumulates in myotubes nuclei in response to TGFβ. Moreover, the silencing of SETDB1 leads to a decrease of pro-fibrotic gene expression in DMD myotubes. Transcriptomic analyses showed a global effect of SETDB1 silencing on the TGFβ pathway response. Interestingly, many TGFβ/SETDB1-dependent genes code for secreted proteins involved in ECM remodelling. Therefore, to test the impact of these secreted factors, we performed conditioned

medium (CM) experiments on myoblasts. These assays revealed that the CM produced by TGFβ-treated myotubes has an inhibitory effect on the receiving myoblasts differentiation. We characterized the composition of the CM by mass spectrometry and we now aim to identify the potential secreted candidates responsible for the observed inhibitory impact on myoblasts differentiation. Moreover, we will perform conditioned medium experiments on fibroblasts, which can differentiate into myofibroblasts, the main actors of fibrosis. This will allow us to decipher the functional significance of SETDB1 silencing on muscle differentiation and on fibroblasts identity and function.

CELL BIOLOGY

#20 Centriole rotational polarity is required for daughter centriole repositioning during primary cilium formation

Juliette AZIMZADEH, Institut Jacques Monod

Centrioles are cylindrical structure essential for the assembly of centrosomes and cilia. The centrosome, composed of two centrioles and a protein matrix, is the main organizer of the microtubule cytoskeleton. In addition, most vertebrate cells possess a primary cilium, a sensory organelle that assembles from the oldest centriole of the centrosome. Centrioles are formed by microtubule triplets in a ninefold symmetric arrangement. In flagellated protists and some animal cells, accessory structures tethered to specific triplets render the centrioles rotationally asymmetric. Centriole rotational asymmetry is required to polarise ciliary beating in these cells. We previously established that rotational asymmetry is also unexpectedly conserved in the centrioles of the human centrosome, despite the lack of asymmetry in appendage distribution in this context. Using expansion microscopy, we found that the protein LRRCC1 is enriched on two triplets in the distal end of centrioles. We now show that CCDC61 localises asymmetrically on the same triplets as LRRCC1, but at their proximal end. CCDC61 depends on LRRCC1 for recruitment to the centrioles and transiently colocalizes with LRRCC1 during centriole assembly. We also identify K1328 as an interactor of CCDC61 that co-localises with CCDC61 at the proximal end of centrioles. We show that CCDC61 and K1328 are both required for proper centriole cohesion in ciliated human cells. One plausible hypothesis is that CCDC61 and K1328 enable the formation of a linker that connects the side of the daughter centriole to the proximal end of the mother centriole in ciliated cells. In possible agreement, we found that CCDC61 inhibits a tubulin modification and the recruitment of pericentriolar matrix proteins to the triplets to which it localises, which could facilitate linker attachment to these same triplets. Taken together, our results suggest that centriole asymmetry in the human centrosome is linked to the assembly of a linker that positions the daughter centriole close to the mother centriole in ciliated cells.

#21 ORP3 and Lipid Transport in Mitosis

Anaïs VERTUEUX, Institut Jacques Monod

Phosphoinositides are lipids with multiple functions. They can recruit cytoskeletal nucleating factors to exert force on their resident membranes. During cell division, phosphoinositides, especially PIP2 form dynamic patterns at the plasma membrane, providing a transmission line to the cytoskeleton, necessary from chromatin segregation to abscission. Multiple kinases and phosphatases remodel phosphoinositides at membranes, partially explaining their regulations. Lipid transport at membrane contact sites (MCS) also contribute to the lipid composition of cell membranes, particularly phosphoinositides. MCS consist of the apposition of membranes from distinct organelles, where lipid

transport proteins foster intermembrane transfer of lipids. However, the regulation of phosphoinositide transport at MCS in cell division remains unexplored. ORP3 is a lipid transfer protein binding PIP2 and transporting PI4P from the plasma membrane (PM) to the ER. ORP3 harbors two binding domains to the ER protein VAPA: a canonical domain (FFAT1) and a phospho-inducible domain (FFAT2). I have shown that ORP3 depletion leads to various mitosis and ploidy defects. During mitosis, the localization of ORP3 is spatially and temporally regulated. ORP3 is mostly cytoplasmic during interphase and shifts to the ER in a VAPA-dependent and FFAT2-phosphorylation manner in prometaphase. ORP3 KO or KD is correlated to chromosome alignment and condensation defects along the mitotic furrow, suggesting a defective distribution of polarity complexes located at the plasma membrane. During telophase, ORP3 is concentrated at the cytoplasmic bridge. It may contribute to PIP2 regulation by depleting PI4P, the PIP2 substrate at the PM. Accordingly, a non functional mutant of ORP3 unable to transport PI4P accumulates at the bridge, suggesting accumulation of PIP2. In conclusion, ORP3 localization regulates PIP2 and PI4P distribution at the PM at different steps during mitosis, which is crucial to allow proper chromosome transmission to the daughter cells.

#22 Impact of RhoA-mediated contractility at the multi-cellular scale in cell extrusion process in MDCK epithelia

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Cell extrusion is one of the mechanisms allowing abnormal or supernumerary cells to be eliminated from epithelia in order to control the integrity of the tissue. Mechanical stresses in epithelial tissue, such as induced by cell compaction or topological defects, have been shown to trigger the initiation of extrusion events. However, the details of decision-making during mechanically-induced extrusions remain poorly known. In particular, why a specific cell is extruded out of a crowded epithelium is still not well understood, neither is the fate of extruded cells. They can activate apoptotic pathways before being removed from the tissue or can be extruded alive: what are the mechanisms that regulate these different outputs ? We address this question through a combination optogenetic and microscopy techniques. Optogenetics approaches are combined with Traction Force Microscopy experiments to take the quantitative aspect of the tool to a higher level. Optogenetics are used in this project as local mechanical force tuners: by controlling RhoA activation and subsequent myosin contraction, they allow us to trigger cell contractility in a local and semi-quantitative manner. the inference of physical parameters such as traction forces associated with our optogenetics system allow us to study precisely the role of mechanical forces in cell extrusion. Combining optogenetics and TFM enabled us to show that enhancing cell contractility by stimulating cells increases rate of extruding cells and that those events involve the neighbouring-less-contractile cells. It also show that mechanically induced cell extrusions have an increased probability to be independent of caspase-3 activation as compared to unstimulated cells. Moreover, these extruded cells are increasingly oriented at the basal side of the tissue and show a delayed onset of apoptosis as compared to apically extruded ones. Altogether, these results show a strong relationship between cell contractility, the apico-basal orientation of cell extrusions and the fate of extruded cells.

EPIGENETICS

#23 SMYD3-mediated methylation of ESCRT-III and its role in abscission

Emmanuelle CEDDAHA, Epigénétique & Destin Cellulaire

The methylation of lysine residues is a key post-translational modification (PTM) mostly studied in the context of histone methylation and epigenetic regulation of gene expression. Beyond its role in regulating chromatin, lysine methylation is emerging as a broad regulator of protein function and cellular process. SMYD3 (SET and MYND domain-containing protein 3) is a lysine methyltransferase (KMT) initially characterized as a histone methyltransferase. However, its cytoplasmic localization suggests that it may also methylate non-histone proteins. SMYD3 contributes to tumor formation in a broad spectrum of tumors with a pro-oncogenic role documented in vivo in mice models. The overexpression of SMYD3 is associated with cancer progression in a wide range of tumors and a significant reduction in overall survival. One unresolved question is how SMYD3 contributes to such a broad spectrum of tumors. This wide pro-oncogenic function of SMYD3 suggests that this protein could regulate a common cellular process, such as cell division, that once altered, contributes to tumor development. In a search for new SMYD3 substrates, we recently discovered that SMYD3, which localizes at the intercellular bridge (ICB) of cytokinetic cells, interacts with a key member of ESCRT-III proteins. ESCRT-III proteins are involved in distinct stages of membrane remodeling and scission in key cellular processes, including the final stage of cytokinesis (abscission), which leads to the physical separation of the two daughter cells. ESCRT-III members are recruited as two ring structures at the midbody and polymerize to form helical hetero-polymer filaments which later constrict at the abscission site thereby promoting abscission. However, little is known about the regulation of ESCRTs by PTMs, apart from phosphorylation. We hypothesize that direct lysine methylation by SMYD3 is key to promote cytokinetic abscission through the recruitment, polymerization and organization of ESCRT-III proteins. We show that SMYD3 localizes at the ICB of cytokinetic cells. We conducted a proteomic screen and identified a key ESCRT-III protein in cytokinesis, as a new interactor and substrate of the SMYD3 enzyme. Through mass spectrometry and mutagenesis approaches, we identified the lysine residues methylated by SMYD3. To assess the dynamic localization of the ESCRT-III protein upon methylation, we showed that silencing SMYD3 leads to the redistribution of the ESCRT-III protein at the midbody. This result suggest that SMYD3-mediated methylation is important for the recruitment and/or polymerization of ESCRT-III along the ICB. Additionally, our results show that SMYD3 depletion delays abscission, and rescue experiments showed that the catalytic activity of SMYD3 is required for successful cytokinesis. Moreover, SMYD3 overexpression accelerates abscission. These results show that SMYD3 regulates the timing of abscission through its methyltransferase activity. We also generated HeLa cell lines expressing WT or non-methylatable (lysine-to-arginine or alanine) ESCRT-III mutants, to evaluate by videomicroscopy the impacts of methylation on the abscission process. To formally demonstrate that SMYD3 regulates the abscission timing via the methylation of this ESCRT-III protein, we will investigate the abscission timing of WT or mutant cell lines upon SMYD3 silencing or ectopic expression, using fluorescent and time lapse microscopy. This project should allow us to understand how methylation affects the spatio-temporal dynamics of ESCRT-III during cytokinesis and promotes cancer through dysfunctional cell division.

#24 DNA methylation protects cancer cells against senescence

Xiaoying CHEN, Université Paris Cité

Aberrant DNA methylation paGern is a hallmark of human cancers, prompting targeting of DNA methylation machinery to be explored as an anti-tumor strategy. DNA methyltransferase 1 (DNMT1) and ubiquitin like With PHD and Ring finger domains 1 (UHRF1) are crucial enzymes for DNA methylation maintenance. Interestingly, UHRF1 is overexpressed in most cancers and is a proven oncogene in vivo. Our previous work revealed that the degree of DNA methylation loss induced by UHRF1 depletion is more severe than DNMT1 depletion in colorectal cancer cells, indicating that targeting UHRF1 may be a more effective approach. We employed the auxin-inducible degron (AID) technology, an advanced chemical/genetic system, to observe the long-term consequences of depleting UHRF1 and/or DNMT1 in cancer cells and deciphered the underlying mechanisms based on bioinformatics tools. We found that chronic DNA demethylation triggers cancer cells to undergo cellular senescence. The loss of UHRF1 results in a more rapid and profound senescence phenotype than DNMT1 loss. Intriguingly, this senescence is not accompanied by DNA damage and functions independently of canonical senescence pathways such as p53 and p16/pRB. Non-canonical pathway investigations revealed that cytosolic p21 accumulation contributes to antiapoptosis during senescence. The suppression of cyclic GMP-AMP synthase (cGAS) also alleviates the senescence-associated secretory phenotype (SASP) and lysosomal activity, and this process is independent of stimulator of interferon genes (STING). Xenograi experiments showed that tumor cells can be made senescent in vivo by decreasing DNA methylation. These findings reveal the intrinsic effects of loss of DNA methylation in cancer cells and have practical implications for future therapeutic approaches.

#25 Histone H3 lysine 9 methylation at the Lamin-Associated Domains impacts nuclear stiffness and cellular biomechanical properties

Silvia COMUNIAN, Epigénétique & Destin Cellulaire

Chromatin serves not only as a storage medium for DNA but also plays a key role in influencing the mechanical properties of the nucleus, both globally and locally. Epigenetic modifications, such as the trimethylation of histone 3 at lysine 9 (H3K9me3), contribute to chromatin condensation within heterochromatin, thereby enhancing nuclear stiffness. The H3K9me3-enriched heterochromatin is predominantly enriched at lamina-associated domains (LADs) and is established by the oncogenic lysine methyltransferases SETDB1 and SUV39H. Nuclear stiffness is a limit for the ability of cancer cells to migrate, deform and metastasize. In non-small cell lung cancer (NSCLC) SETDB1, but not SUV39H, is overexpressed and impacts the heterochromatin disposition in the nucleus. However, we found an interplay between SETDB1 and SUV39H in the formation of H3K9me3-heterochromatin at LADs in NSCLC. SETDB1 overexpression in NSCLC inhibits the SUV39H-established H3K9me3 at the LADs. The absence of this “ring” of H3K9me3-enriched heterochromatin at the nuclear periphery reduces the overall cell viscosity (as measured by microfluidics) and the nuclear envelope rigidity (as measured by optical tweezers assay).

#26 Unraveling the functions and mechanisms of epigenetic switching in mammals

Teresa URLI, Institut Jacques Monod

In mammals, 5-Cytosine DNA methylation (5mC) is mostly found in the CpG dinucleotide context. At CpG-rich regulatory elements, such as CpG island (CGI) promoters, 5mC is associated with stable gene

repression. However, the majority of CGI promoters remain protected from 5mC, and gene silencing is typically regulated by the polycomb group (PcG) proteins. Incidentally, 5mC and the PcG-deposited H3K27me3 mark are mutually exclusive at CpG-rich regions of the genome. Interestingly, both marks undergo a massive reorganization during early mammalian embryogenesis. In the naïve pluripotent cells of the blastocyst stage, the genome exhibits low 5mC levels, while H3K27me3 is broadly distributed. As the embryo transitions to the primed epiblast state, when embryonic 5mC is established, the PcG landscape becomes largely restricted to 5mC-free CGIs. Coordination of 5mC and PcG is essential for development, as exemplified by embryonic lethality in mutants for both pathways. A dynamic in cellula differentiation system from naïve mouse embryonic stem cells (ESCs) to primed epiblast-like cells (EpiLCs) recapitulates the epigenome remodeling that occurs in mammalian embryogenesis. Employing a DNA methyltransferase (Dnmt) triple knock out (TKO) line devoid of 5mC as a control, we discovered 1301 SWitch Regions (SWRs), where 5mC displaces H3K27me3 during normal WT differentiation. Notably, we also found 163 loci where the switch leads to gene activation, revealing an emerging role for 5mC in genome regulation. This project investigates this novel mode of gene regulation, focusing on the antagonism between 5mC and PcG. Employing precise epigenome editing we demonstrated the locus-specific regulatory impact of 5mC. We are also interested in the mechanistic aspects of the epigenetic switch from PcG to 5mC and wish to interrogate the molecular basis of their antagonistic relationship. Publicly available data suggests SWRs frequently overlap with the binding of PcG ring finger 6 (PCGF6, PRC1.6 subunit), known to be involved in the PcG-to-5mC switch at a subset of germline-expressed genes. However, in this case, switching to 5mC does not lead to a transcriptional change as CGI promoters gain dense 5mC. I am exploring the hypothesis that PRC1.6 can be a precursor to 5mC deposition, leading to gene activation in some contexts.

#27 The Lonely Pipette podcast; helping scientists do better science

Jonathan WEITZMAN, Epigénétique & Destin Cellulaire

The Lonely Pipette is a month podcast presenting long-format discussions with scientists around the world. The hosts are Jonathan, a professor of genetics and epigenetics and team leader, and Renaud, an independent science communicator. Renaud and Jonathan talk to inspiring people to learn about their habits and recommendations for working scientists. The episodes are in English and are freely available on most podcast platforms. Created in autumn 2020, the podcast was well-received by the international life sciences community with more than 85 000 downloads. The podcast has listeners on all continents (more than 140 countries) and was ranked in the top 20 European life science podcasts (2020 and 2021). We will discuss the podcast creation and podcast format. We are always interested in hearing from listeners about what they like and suggestions for future guests.

Don’t stay alone, come join The Lonely Pipette community!

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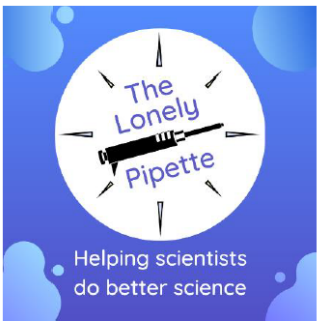

BIOPHYSICS

#28 Intercellular stress anisotropy as a regulator of cell proliferation in epithelia

Lucas ANGER, Institut Jacques Monod

Cell division is one of the most basic process involved in building a complex living organism, and dysfunction of cell division usually leads to severe tissue pathology, during both development and homeostasis. Although the biochemical regulation of the cell cycle has been extensively studied, various works in the field of mechanobiology have also shown how tissue mechanics can affect the spatio-temporal organization of cell division. Due to the lack of direct intercellular mechanical stress measurements, how intercellular deformations impact cell division fate remains unclear, but the widely accepted process is that local tension can induce cell division. Here, building on the framework of continuum mechanics, we show that the mechanical stress within epithelial monolayers exhibits a principal stress direction, revealing the highly anisotropic nature of epithelia as a soft material, regardless of the cell density. Using traction force microscopy and stress inference technique, we demonstrate that increased anisotropic stress causes cells to divide along the principal stress direction, and that cell division allows anisotropic stress within the tissue to decrease. We show that the anisotropic stress is a mechanical predictor of cell division, covering the usual view (tension-induced division) as well as non-trivial cases (division happening under compression or no clear mechanical state). Our findings indicate a mechanical feedback loop between tissue homeostasis and intercellular stress anisotropy in epithelia, and suggest broad implications in development and in pathology involving unregulated division.

#29 A collection of experimental methods for mechanobiology

Nathan MARTIN-FORNIER, Institut Jacques Monod

The History of Biology and Medicine are inextricably linked with that of the microscope. As such, most commonly, the study of living objects has relied on pure observation, and interactions with the samples are often limited to being mostly chemical (using drugs or biochemical agents) or electromagnetic (irradiation, photo-activation…). Nevertheless, the past decades have shown that living organisms are also very physical objects, that can often mechanically sense and interact with their surroundings, and understanding this aspect proved to be essential in several fields of study such as tissues and organ growth, morphogenesis, nuclear dynamics, and the study of diseases such as Alzheimer and cancers. For this reason, in the context of the BioMechanOE project of the Labex Who am I? which includes teams from the Matière et Systèmes Complexes (MSC) and the Institut Jacques Monod (IJM) laboratories at Université Paris Cité, we have developed several setups in order to apply and measure different ranges of forces at various length and timescales, to a wide array of biological systems. These setups include optical tweezers, glass microplates, micropipettes, magnetic stretcher, indentation systems… These methods, potentially combined with other microscopy techniques such as confocal imaging, FRET, FLIM, optogenetics, etc. allow us to monitor the state of living systems under controlled constraints, both biological and mechanical in nature.

#30 Regulation of neuroepithelium mechanics by PAX3 transcription factor in organoids

Camil MIRDASS, Institut Jacques Monod

Myelomeningocele, also termed spina bifida, is a frequent congenital malformation characterized by incomplete closure of the caudal neural tube during embryogenesis. Neural tube closure is a well-documented and intricate morphogenetic and mechanical event finely regulated in space and time. However, it is still poorly understood how genetic aberrations found in patients disrupt neural tube closure mechanics and lead to myelomeningocele emergence. Here we focus on PAX3, a transcription factor whose loss-of-function is associated to myelomeningocele in humans, and show that PAX3 loss results in severe neuroepithelium morphogenesis phenotypes in a spinal organoid model. We found that the absence of one or two PAX3 allele leads to altered epithelial structures within organoids with disrupted cellular arrangements, a fragmented extracellular matrix deposition and a compromised accumulation of apical polarity markers for cells on organoid periphery. These morphogenetic phenotypes correlated with the differential expression of several regulators of cell adhesion and cytoskeleton remodelling as well as glucose metabolism whose involvement in embryonic morphogenetic events is being increasingly described. We also revealed that complete loss of PAX3 induces a reduction of chromatin openness in regulatory regions near genes governing epithelial structure. Furthermore, in order to better unveil epithelial mechanics and junction dynamics, we established a 2D neuroepithelium culture assay from dissociated organoids. Although preliminary, PAX3 loss disrupts cytoskeleton organization with a thicker actin cortex network and induces adherent junction relocalization from tricellular junctions along cell boundaries. Our results unveil a novel role for PAX3 in the regulation of mechanical processes underlying neuroepithelium morphogenesis in organoids, hence highlighting the interplay between transcription regulation and mechanics in neural tube closure.

#31 Investigating the mechanical cross-talk between mitochondria and the cytoskeleton

Emilie SU, Matière et Systèmes Complexes

Widely acknowledged as the cellular energy powerhouses, mitochondria are dynamic organelles capable of sensing and integrating mechanical and metabolic cues. Previous studies have examined the influence of external forces applied to the cell on mitochondria mechanical response [1], but a direct assessment of their mechanical properties within living cells is still lacking. Moreover, although the role of the cytoskeleton in regulating mitochondria morphodynamics has received considerable attention [2, 3], the mechanical cross-talk between mitochondria and the cytoskeleton remains under investigation. Here we have used an optical tweezers-based intracellular micromanipulation technique with endocytosed beads [4], to probe the mechanical and rheological properties of single mitochondria and their microenvironment in RPE-1 cells. Our novel approach involves applying controlled forces to deflect single mitochondria, enabling the direct measurement of their effective stiffness. Additionally, we performed oscillatory intracellular rheology and selectively disrupted the actin and microtubule cytoskeletons to investigate their role in the mitochondrial microenvironment. Our results reveal that the viscoelasticity of the cytoplasm is correlated with the local density of mitochondria only when actin is depolymerized and not in control conditions or when microtubules are disrupted. This indicates that actin filaments play a more significant mechanical role than microtubules in the mitochondrial microenvironment, at this spatio-temporal scale. Further experiments were conducted to assess the mechanical interplay between mitochondria and the cytoskeletal components, by quantifying force

transmission through oscillations within the mitochondrial network. Our findings demonstrate that the actin cytoskeleton is the main mediator of force transmission to mitochondria. Further experiments focusing on the actomyosin contractility should provide valuable insights into the underlying mechanisms.

#32 Microfluidic platform for investigation of intestinal epithelial interaction with bacteria

Jasmina VIDIC, INRAE

Campylobacter jejuni is the most common cause of foodborne gastroenteritis in humans with about 550 million annual infections worldwide. The extracellular vesicles (EVs) of C. jejuni have an important impact during pathogenicity but their role in invasion of the host intestinal epithelial cells remains largely unknown. In vitro models lack the complexity of tissue and fail to replicate the dynamic interactions between EVs and human intestinal epithelial cells accurately, while animal infection models bring ethical concerns. To bridge this gap, we propose a microfluidic platform integrated with an impedimetric sensor for real-time monitoring of C. jejuni EVs interaction with human intestinal epithelial cells. When cultured in this microfluidic device, Caco-2 epithelial cells underwent 3D morphogenesis and spatially organized in spheroid-like structures. Functional assays revealed that C. jejuni secretome and EVs have a significant cytotoxic effect on Caco-2 cultured on plates. However, 3D Caco-2 spheroids showed increased resistance to the toxicity of secreted virulence factors of C. jejuni. By combining the impedance spectroscopy and live cell imaging, the platform allowed real-time monitoring of cellular spatial growth and sensitive detection of the EVs ability to reach and damage intestinal epithelial cells organized in 3D morphologies. Thus, the developed microfluidic device offers a promising platform for investigating host-microbe interactions, and may have a broad impact on biomedical research of gastroenteritis.

#33 Morphogenesis of the gastrovascular canal network in scyphomedusae: Variability and possible mechanisms

Stanisław ŻUKOWSKI, Matière et Systèmes Complexes

Scyphomedusae are free-swimming medusae which are part of the phylum Cnidaria, the sister group to all animals with bilateral symmetry. Scyphomedusae possess a gastrovascular system with a canal network distributing nutrients and oxygen to tissues in the subumbrella. A large diversity of canal networks exists, from highly reticulated networks in Rhizostoma pulmo to purely branching networks in Cyanea capillata. The canal network of Aurelia jellyfish has a sparse reticulated network. By day-to-day macroscopic observations, we study the dynamics of the network formation in juvenile Aurelia jellyfish and model it numerically. During the network development, at the circular canal at the rim of the jellyfish umbrella, an instability emerges in form of sprouts. They then grow toward the center of the jellifish and reconnect to already existing radial canals. These reconnections have a bias to reconnect to the younger side radial canal. However, even in clones, there exists a variability towards which canal the reconnections occur. Similar to the morphogenic instability idea of Turing (1952), the canal network pattern is not strictly regulated, but rather grows from an instability, keeping trace of noise, and then self-organizes, guided by physical rules. We show that in Aurelia both the hydrodynamic effects, such as pressure in the canals, and elastic effects, such as deformation of the jellyfish body during swimming, govern the direction toward which the canal sprouts grow. We suggest that these morphogenic instabilities also play a role in the diverse patterns of canal networks in Rhizostoma pulmo and Cyanea capillata.