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FIFTEEN YEARS OF EPIGENETICS & CELL FATE

ABSTRACT BOOK

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Talks

Posters

Edith Heard : Life with two X chromosomes

EMBL director, Heidelberg, Germany.

X-chromosome inactivation (XCI) is a process that silences one of the two X chromosomes in female mammals leading to stable and clonally heritable gene repression early on in life. This process is a paradigm for epigenetics and it ensures appropriate dosage for X-linked gene products in XX females, compared to XY males who only have one X chromosome. Without XCI, an XX female embryo would die. Thanks to X inactivation, most females are cell mosaics when it comes to expression of their paternal or maternal X- chromosome genes. This has important implications for female physiology and disease. Furthermore, some genes can escape from silencing on the inactive X and this can also impact female cellular and tissue functions and influence onset of autoimmune and other pathologies. Understanding the basis for sex bias in disease is now recognised to be of social and economic importance. For centuries, female biology and medicine had been understudied for historical and sociological reasons. Yet sex is a key determinant of physiology, anatomy, and plays a major role in the differential manifestation of various diseases, affecting both clinical outcome as well as response to therapy. Both hormonal and genetic factors contribute to this sex bias, and environmental components can also influence disease onset.

Our work has led us to think about the specificities that the presence of the inactive X chromosome might have for female development and disease and the evolutionary implications of these specificities.

Jérôme Dejardin : Deciphering telomere looping functions

Institute of Human Genetics CNRS-Université de Montpellier UMR 9002, Montpellier, France.

Chromosome-end protection is a crucial aspect of genome stability. Telomere-loops (t-loops) are believed to protect telomeres, yet direct evidence for this role has remained elusive. In most differentiated cell types, t-loop stabilization relies on the shelterin protein TRF2, while in pluripotent cells, the regulatory pathways remain undefined. We have identified Telomere Loop Stabilizing Protein 1 (TELS1), which stabilizes t-loops independently of TRF2 in pluripotent cells. TELS1 binds to single-stranded G-rich telomeric DNA and facilitates ssDNA strand invasion into duplex DNA in vitro, suggesting a direct role in t-loop stabilization. In pluripotent cells, shelterin-proficient telomeres without t-loops remain protected but become more accessible to telomerase, leading to telomere lengthening. In differentiated cells, where t-loop formation depends on TRF2, TELS1 can effectively substitute for TRF2 to stabilize t-loops. T-loop rescue in this shelterin-deficient context mitigates but does not entirely suppress checkpoint activation and subsequent telomere fusions. Our findings suggest that t-loops contribute to telomere length homeostasis and confirm their role in telomere integrity, but they are neither solely necessary nor fully sufficient for complete telomere protection.

Justine Marsolier: Understanding & fighting drug persistence in TNBC

CNRS UMR3244, Institut Curie, PSL University, Paris, France.

Emergence of resistant cancer cells to anti-cancer therapies is a multistep process including the survival of a subpopulation of cells to the drug insult. These cells are tolerant/persister cells and constitute a reservoir of cells from which drug-resistant cells, which can actively grow under treatment, ultimately emerge. Preventing the transition to the persister state is now one promising therapeutic strategy to prevent drug resistance but detailed insight on the mechanisms driving the emergence of persister cells is essential to design efficient therapeutic approaches.

In several studies in different cancers upon targeted or chemotherapies, genetic and/or non-genetic alterations have been proposed to drive drug tolerance and resistance. In a previous study in the team of Céline Vallot, we built the identity cards of persister cells in TNBC - Triple Negative Breast Cancer - through a complete characterization of their transcriptomic and epigenomic features at the single cell resolution. We demonstrated that the repressive histone mark H3K27me3 regulated gene expression and cell fate at early stages upon drug treatment. Indeed, upon therapeutic stress, recurrent and organized loss of H3K27me3 launched the expression of a set of persister genes and enhanced the potential of each cancer cell to tolerate chemotherapy. The persister expression program included genes defining the mammary basal cell identity, several markers of EMT - Epithelial to Mesenchymal Transition - and genes controlling cell metabolism. Now, we want to uncover the molecular mechanisms driving the activation of the persister expression program over the course of anti-cancer treatment focusing (i) on the predicted master transcriptional factor complex AP-1 and (ii) the epi-regulators of H3K27me3. Overall, deciphering molecular mechanisms driving cancer cell response to therapeutic stress is urgently mandatory to design new relevant TNBC standard of care to prevent recurrence in this sub-group of very aggressive breast cancer which remains the main challenge nowadays to patient full cure.

Souhila Medjkane : Methylation regulates the timing of cytokinetic abscission

Epigenetics and Cell Fate Centre, CNRS, Université Paris Cité, Paris, France

Lysine methylation is a key post-translational modification, mostly studied in the context of histone methylation and epigenetic regulation of gene expression. However, non-histone protein methylation is emerging as a regulator of broader cellular processes. SMYD2 is a lysine methyltransferase, which contributes to cancer progression *in vivo* in mouse models and its overexpression is associated with a significant reduction in overall survival and cancer progression in a wide range of tumors.
We discovered that SMYD2 methylates human CHMP2B, a key effector of cytokinesis, that cleaves the intercellular bridge connecting two daughter cells. We demonstrated the functional role of CHMP2B methylation in actively promoting abscission by facilitating the repositioning of ESCRT-III proteins at the cleavage point. Our findings extend beyond cytokinesis, as CHMP2B methylation also facilitates HIV-1 virus budding, suggesting a broader involvement in ESCRT-dependent cellular processes.

Antonin Morillon: Beyond fantasy, the cryptic dark transcriptome represents an unexplored source of tumor specific antigens

NcRNA, Epigenetic and Genome Fluidity, CNRS UMR3244 Sorbonne University, PSL University, Institut Curie, Centre de Recherche Paris France

Long non-coding RNAs (lncRNAs) are involved in regulating various cellular processes. While initially thought to lack coding potential, recent studies have shown that some lncRNAs can be translated into lncRNA-derived peptides. Despite the growing interest in these peptides, their potential functions and the mechanisms governing their synthesis remain largely unexplored. Here, we investigated the functional impact of non-canonical translation events on cytoplasmic lncRNAs in human cells.

We have recently shown that Xrn1-sensitive cytoplasmic lncRNAs (XUTs) in yeast are translated even in NMD-competent cells, suggesting that despite the cryptic nature of the transcripts, their translation result in detectable products. In human cells, we identified DIS3, and not Xrn1, as the main exonuclease restricting accumulation of lncRNAs in the cytoplasm and revealed thousands of DIS3-sensitive lncRNAs (DISTs). We show that DISTs also display active translation, producing peptides predicted to be high-affinity antigens in multiple myeloma patients carrying DIS3 mutations. Finally, immunogenic tests revealed that the resulting neoAntigens can be recognized by T cell collected from blood, opening new strategies for the next generation of immunotherapies. Overall, our work highlights the central role of translation in the metabolism of cytoplasmic lncRNAs, with different potential outcomes. While the resulting peptides could constitute raw material exposed to the natural selection in yeast, we propose that some could be part of the cell-to-cell communication through tumor-specific antigen presentation in human cells.

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Chiara Mozzetta : Peripheral heterochromatin's role in genome integrity and cell identity of multipotent stromal cells

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Spatial genome organization is essential for maintaining proper gene expression patterns in a cell-type-specific manner. Silent heterochromatin is localized at the nuclear periphery and anchored to the nuclear lamina (NL) by lamina-associated domains (LADs), embedding alternate fate genes to preserve cell identity. However, the inheritance of heterochromatic lamina-associated regions after cell division and the potential disruption of peripheral heterochromatin organization as a precursor to malignant cell transformation remain poorly understood. Here, we will share our latest findings on the role of peripheral heterochromatin in preserving genome integrity and cell identity in skeletal muscle-resident multipotent stromal cells (MSCs). Building on our recent study that identified Prdm16 as a MSCs-specific regulator of heterochromatin assembly at the nuclear envelope, we will present data demonstrating its role in mediating LAD deposition and maintaining nuclear architecture and genome stability. We will provide evidence showing that the absence of Prdm16 in MSCs leads to genomic abnormalities by disrupting stable LAD-NL interactions during cell division, resulting in mitotic errors. Finally, we will discuss how the MSC-specific loss of Prdm16 may predispose these mesenchymal progenitors to acquiring (pre)-malignant features.

Charbel Alfeghaly : Unraveling the multifaceted activities of XIST during early human development

Epigenetics and Cell Fate Centre, CNRS, Université Paris Cité, Paris, France

In placental mammals, the equalization of most X-linked gene products between females and males is ensured through X-chromosome inactivation. In contrast to mouse, the master regulator XIST is expressed early during human preimplantation development and accumulates on both X-chromosomes without triggering X-inactivation. Previous studies reported a transient form of dosage compensation in female human preimplantation embryos, which occurs through the dampening of X-linked gene expression from both X-chromosomes. However, the existence of X-chromosome dampening (XCD) and whether it is mediated by XIST remained controversial. Hence, we set out to explore the role of XIST and its implication in XCD in human preimplantation development, by using naïve hESCs as a cellular model. We show that XIST contacts the majority of the active X chromosome and triggers the deposition of Polycomb-mediated repressive histone modifications. Furthermore, we reveal that XIST dampens the transcription of most X-linked genes through interactions with specific protein partners involved in transcriptional repression. Our study demonstrates that XIST is functional before XCI, confirms the existence of a transient process of X chromosome dosage compensation and reveals that XCI and XCD rely on similar set of factors.

Rebecca Oakey : Epigenetic regulation of host/nested gene pairs: implications for development and disease

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Genomic organisation is complex and in mammals the majority of genes are organised singly, however, about a sixth of human (17%) and a tenth of mouse (12%) genes harbour at least one other gene. The configuration of two genes contained one within the other is known as a host/nested gene pair. Genes organised like this can cooperatively regulate one another because they occupy the same genomic space. Nested gene expression can impact host gene pre-mRNA processing and generate host RNA isoforms with different stability and coding capacity. This occurs at known imprinted gene loci, where the expression of a nested gene is associated with alternative polyadenylation site use by the host gene and its silencing with canonical polyadenylation site use. This type of genomic organisation and isoform diversity extends beyond the imprinted gene context, occurring at host genes harbouring nested LINE1 retrotransposons, nested genes or putative intragenic promoters Growing evidence indicates that short isoforms generated through alternative polyadenylation are key for cellular functions and have implications for tissue specific gene regulation in development. The genetic and epigenetic mechanisms involved in host/nested gene expression are being interrogate. Specifically, the impact of transcription, the impact of polyadenylation site deletions using CRISPR Cas9 editing tools and the role of chromatin state and of the binding of trans-acting factors are providing clarity on the mechanisms coordinating host/nested gene pair expression.

Beatrice Rondinelli : Investigating new functions of chromatin-related enzymes in the DNA Damage Response

Genome Integrity and Cancers UMR9019, CNRS and Gustave Roussy Institute, Villejuif, France

Our team has a long-standing interest in studying how chromatin organization and function protect the mammalian genome from harmful lesions by participating to the DNA Damage Response (DDR). As a model, we study how cancer-associated chromatin alterations (through mutations or aberrant expression) contribute to the onset of genome instability, an enabling characteristic of cancer. Our research has two main goals: i. to dissect new chromatin-related mechanisms of genome integrity maintenance, many of which still lay uncovered, ii. to identify dysfunctions of these processes in human cancer, which may represent cellular vulnerabilities and thus turn into prognostic markers or therapeutic targets to develop in order to improve patients' response to treatment.
Here, I will present one of three projects currently ongoing in the lab, that started in May 2022. By analyzing available pharmacogenomic and proteomic datasets, we identified some chromatin-related enzymes that participate to the DDR and genome integrity maintenance in human cells. We are currently investigating their uncharacterized functions by means of imaging, cellular and proteomic approaches and by exploiting loss-of-function and gene edited cellular models.

Dounia Djeghloul : Mitotic inheritance of cell identity and epigenetic memory

Epigenetics and Cell Fate Centre, CNRS, Université Paris Cité, Paris, France

Understanding how cell identity and epigenetic information are preserved through cell division is a central challenge in biology with key implications for health and disease. Cell-specific transcription program and chromatin state are faithfully transmitted from mother to daughter cells as they divide. In mitosis, while many DNA-associated factors are displaced from condensed chromosomes, others remain bound to "bookmark" the genome, ensuring accurate conveyance of cell identity. To address this, I developed novel technologies to quantify and functionally probe factors bound to native (unfixed) mitotic chromosomes. Notably, this work led to identify a crucial role for histone H3K9 methylation (a heterochromatin mark) in sustaining mitotic chromosome structure and bookmarking in embryonic stem cells (ESCs). The concept of bookmarking comes mainly from studies in ESCs, which are short-lived *in vivo*. These mechanisms may differ in committed cells that require stable maintenance of lineage identity throughout life. Additional studies are thus needed to understand cell identity inheritance during lineage differentiation.

B lymphocytes arise from hematopoietic stem cells through a succession of lineage restriction, determination and specialization programs. Stable maintenance of B lineage identity is essential for B cell production and accurate immune function. My goal is to use the convergent approaches I developed, in order to study mechanisms by which B lineage identity is inherited and its impact on the immune system. First, we will undertake a comprehensive analysis of mitotic chromatin-bound factors at different stages of B lymphoid differentiation then investigate the role of these factors in conveying B lymphoid cell identity and function. Additionally, we will analyse the epigenetic characteristics of mitotic chromosomes and assess the role of heterochromatin in sustaining B lineage identity. The third axis of my research focuses on X chromosome inactivation (XCI), a paradigm of epigenetic inheritance, in human female B lymphocytes. The X chromosome is enriched for immune genes and XCI maintenance is essential for normal immune pathways. We will assess the mitotic proteome of active and inactive X chromosomes, and test the ability of newly-identified candidates to maintain silencing of X-linked immune genes. Lastly, we will explore the impact of heterochromatin changes on XCI mitotic memory and increased escape of immune genes that may underpin female-biased immune response.

Julie Chaumeil : Sex bias in immunity: X chromosome inactivation and autoimmunity

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Biological sex influences physiological differences between males and females and affects the prevalence of many diseases. While the roles of sex hormones have been well documented, recent studies are shifting toward understanding the genetic impacts of sex (XX or XY chromosomes). The immune compartment represents a model of choice to investigate this question. The sex-based differences in immune challenges include a greater immune response against pathogens and stronger responses to vaccination for women than men. However, it seems to come with a price, with women more at risk for some autoimmune diseases, including systemic lupus erythematosus (SLE). A growing body of data shows that not only sex hormones but also sex-chromosome associated loci play a role in the physiological and pathological immune pathways. This is particularly clear for TLR7, a single-stranded RNA receptor encoded by an X-linked gene. The response initiated by TLR7-mediated sensing of ssRNA by innate immune cells and B cells is an essential line of defense against RNA viruses. However, TLR7 can also respond to endogenous ssRNA-containing ligands, potentially leading to autoimmunity or inflammation if not properly controlled. Deletion of *Tlr7* reduces sex differences in vaccine-induced antibody responses and protection, while expression of 2 copies of the *Tlr7* gene in mice is sufficient to induce systemic autoimmunity. In female mammals, one of the two X chromosomes is randomly inactivated to equalize the dosage of X-linked gene expression between sexes. This X-chromosome inactivation (XCI) is established at an early stage of female embryogenesis and results in cellular mosaicism. However, in both Human and mouse, some X-linked genes escape XCI in certain tissues or individuals and are thus expressed from both the active (Xa) and inactive (Xi) X chromosomes (around 5% in female mice, up to 23% in women). The importance of tight regulation of *TLR7* expression led to the hypothesis that escape from silencing by incomplete XCI in female immune cells may affect TLR7-signaling resulting in spontaneous triggering of harmful autoreactive responses leading to disease. Indeed, with the lab of Jean-Charles Guéry (INFINITY, Toulouse) we showed that *TLR7, but also TLR8,* can escape XCI in a fraction of immune cells from women leading to a greater dose of TLR7 or TLR8 protein. Strikingly, B cells expressing *TLR7* biallelically were more responsive than monoallelic cells at specific checkpoints of B cell differentiation that involve signaling through TLR7. We are now trying to dissect the mechanisms underlying the escape of these genes from XCI with an emphasis on the 3D organization of the *TLR7/8* genomic region with respect to the X chromosome territory.

Rachid Elfatimy: A nuclear function for an oncogenic microRNA as a modulator of splicing

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miRNAs are regulatory transcripts established as repressors of mRNA stability and translation that have been functionally implicated in carcinogenesis. miR10b is one of the key oncomiRs associated with multiple forms of cancer. Malignant gliomas exhibit particularly striking dependence on miR10b. However, despite the therapeutic potential of miR10b targeting, this miRNA's poorly investigated and largely unconventional properties hamper the clinical translation. We utilized Covalent Ligation of Endogenous Argonautebound RNAs and their highthroughput RNA sequencing to identify the miR10b interactome and a combination of biochemical and imaging approaches for target validation. We demonstrate that miR10b binds to U6 snRNA, a core component of the spliceosomal machinery. We provide evidence of direct binding between miR10b and U6, in situ imaging of miR10b and U6 colocalization in glioma cells and tumors, and biochemical co-isolation of miR10b with the components of the spliceosome. We further demonstrate that miR10b modulates U6 N6 adenosine methylation and pseudouridylation, U6 binding to splicing factors SART3 and PRPF8, and regulates U6 stability, conformation, and levels. These effects on U6 result in global splicing alterations, exemplified by the altered ratio of the isoforms of a small GTPase CDC42, reduced overall CDC42 levels, and downstream CDC42 mediated effects on cell viability. In conclusion, we identified U6 snRNA, the key RNA component of the spliceosome, as the top miR10b target in glioblastoma. We, therefore, present an unexpected intersection of the miRNA and splicing machineries and a new nuclear function for a major cancer associated miRNA.

Silvia Comunian : Histone H3 lysine 9 methylation at the Lamin-Associated Domains impacts nuclear stiffness and cellular biomechanical properties

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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).

Our results show an interplay between SETDB1 and SUV39H in the regulation of nuclear mechanical properties in lung cancer cells, unraveling a non-conventional role of epigenetic mechanisms in nuclear rigidity of cancer cells.

Poster n°1: EZHIP maintains parental H3K27me3 asymmetric distribution and non-canonical imprinting in mouse embryos

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Parental epigenomes are very distinct in early mammalian embryos, and their remodelling is essential for development. A paradigmatic example is the distribution of trimethylation of histone H3 lysine 27 (H3K27me3), the mark deposited by the Polycomb repressive complex 2 (PRC2): at fertilization, it is mostly depleted from the paternal genome, while it is abundant on the maternal genome. Recent studies have revealed that in mouse, maternal H3K27me3 underlies non-canonical (DNA methylation independent) genomic imprinting and is crucial for embryonic viability. Here, we demonstrate that maternal EZHIP, a cofactor of PRC2, is crucial for the maintenance of H3K27me3 parental asymmetry during early development by preventing aberrant gain of H3K27me3 over the paternal genome. Surprisingly, at non-canonical imprinted genes, enrichment of H3K27me3 on both alleles is associated with biallelic transcription at the time of implantation, and this loss of imprinted expression remains for a subset of genes in extraembryonic lineages after implantation. The *Xist* long noncoding gene is amongst the non-canonical imprinted genes that are affected, and we show that its ectopic maternal expression leads to aberrant X chromosome inactivation, and to reduced embryonic viability. Our results not only unveil EZHIP as a new player for the dynamic regulation of H3K27me3 in early embryonic development, but also identify a role for the parental asymmetry of H3K27me3 in non-canonical imprinting mechanisms.

Poster N° 2: Parasite protein secretion takes the reins of bovine gene expression

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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.

Poster N° 3 : enSCORE platform : Engineering organoids since 2021

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[1] enSCORE platform – Université Paris Cité

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 platformworks 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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Poster N° 4: Identification and characterization of new substrates of the lysine methyltransferases SMYD2 and SMYD3 involved in tumor progression

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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.

Poster N° 5: SMYD3-mediated methylation of ESCRT-III and its role in abscission

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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 impacts the spatio-temporal dynamics of ESCRT-III during cytokinesis and promotes cancer through dysfunctional cell division.

Poster N° 6: DNA methylation protects cancer cells against senescence

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Inhibitors of DNA methylation such as 5-aza-deoxycytidine are widely used in experimental and clinical settings. However, their mechanism of action is such that DNA damage inevitably co-occurs with loss of DNA methylation, making it challenging to discern their respective effects. Here we deconvolute the effects of decreased DNA methylation and DNA damage on cancer cells, by using degron alleles of key DNA methylation regulators. We report that cancer cells with decreased DNA methylation —but no DNA damage— enter cellular senescence, with G1 arrest, SASP expression, and SA-β-gal positivity. This senescence is independent of p53 and Rb, but involves p21, which is cytoplasmic and inhibits apoptosis, and cGAS, playing a STING-independent role in the nucleus. Xenograft experiments show 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.

Poster N° 7: Distinct functions of Krebs cycle metabolites in modulating stem cell survival and mitochondrial dynamics

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The conversions of the metabolic demands between glycolysis and oxidative phosphorylation remodel embryonic stem cell (ESC) pluripotency and differentiation. Metabolites can impact cell fate through epigenetic landscapes such as TCA cycle metabolite α-ketoglutarate promotes histone and DNA demethylation in maintaining pluripotency. However, whether the other Krebs cycle metabolites can affect pluripotent status have not been comprehensively characterized yet. Among the eight Krebs cycle metabolites, we identified citrate, succinyl CoA, oxaloacetate and malate led to pluripotent human ESC death. Isocitrate strikingly promoted ESC differentiation and rescued the cellular death caused by oxaloacetate in ESC and in mouse early embryos. *In vitro* manipulation of the exit of ESC pluripotency, mitochondria changed from punctate into the filamentous network. The differentiated hESC and isocitrate treatment counteracted the oxaloacetate-caused cell death by restoring cristae structure and elongating mitochondrial configuration. These results suggest that mitochondrial membrane structures and dynamics remodeled during the exit of pluripotency and Krebs cycle metabolites have distinct functions in modulating stem cell pluripotency and survival.

Poster N° 8: Facultative heterochromatin maintenance in response to DNA double-strand breaks in mammalian cells

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In mammals, the DNA damage response takes place in chromatin and triggers profound chromatin alterations. Facultative heterochromatin regions are characterized by their enrichment in H3K27me3, an epigenetic mark playing a key role in transcriptional silencing and cell type specification. However, the maintenance of H3K27me3 is challenged during DNA repair and it is unclear how this mark is regulated in response to DNA damage and with what consequences on genome/epigenome integrity.

To decipher the chromatin changes that accompany DNA double-strand break (DSB) repair in facultative heterochromatin, we combine DSB induction with imaging, proteomics and next-generation sequencing in human cells. We show that H3K27me3 remains stable at DSBs despite the recruitment of the cognate methyltransferase EZH2, and we dissect the underlying mechanisms and the functional relevance of H3K27me3 stability for genome maintenance. By targeting the Cas9 nuclease to genomic regions enriched in H3K27me3, we analyze the genomic instability profiles and explore the impact of H3K27me3 deregulation on DSB repair pathway choice. In parallel, we profile facultative heterochromatin marks in the vicinity of DSBs to assess the impact of repair on heterochromatin maintenance. Together, this work should shed light on the interplay between the DSB response and facultative heterochromatin states.

Poster N°9 : Exploring the role of *XIST* and X chromosome inactivation in pre-implantation development using human embryoid models

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In all eutherian mammals, X-linked gene dosage is balanced between the two sexes (XX, XY) early during embryonic development, through the almost complete transcriptional inactivation of one X chromosome in the female. Decades of studies in mouse have established that the lncRNA *Xist* acts as a cis-acting master regulator of X chromosome inactivation (XCI). *Xist* is able to coat the X chromosome and initiates a downstream cascade of chromatin modifications leading to the transcriptional inactivation of the coated chromosome. In the absence of *Xist* expression, X dosage is imbalanced and female mouse embryos fail to properly develop, showing severe defects in extraembryonic tissue differentiation. The uncovered link between extraembryonic lineage commitment and XCI has however remained unexplored in human, and species-specific aspects of embryonic development challenge the possibility of translating to human the accumulating evidence in mouse regarding both cell fate commitment and early X chromosome dynamics.
My project aims to explore the significance of X-linked gene regulation in human pre- to early post-implantation time frame, by describing (1) how XCI is established in presumptive embryonic and extraembryonic lineages, and (2) the significance of *XIST* expression for X-linked dosage compensation and proper fate specification, with particular attention given to the trophectoderm extraembryonic lineage. Human embryonic stem cells (hESCs) in a naïve-like state of pluripotency represent the ideal model of a pre-XCI state amenable to provide both embryonic and extraembryonic derivatives *in vitro*, either individually or, as recently discovered, organized in 3D embryoid models called “blastoids”. Using CRISPR-mediated *XIST* repression in 2D differentiation, we recently uncovered a crucial role for XIST in the establishment of XCI in humans and in the maintenance of extraembryonic cell fate. This paves the way for functional exploration of XCI dynamics and requirement in human early development using 3D stem cell-based embryoid models.

Poster N°10 : Transposable element repression: their interconnections and impact on cell fate

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Transposable elements (TEs) occupy half of the mammalian genome, compared to 2% of protein-coding genes. Though TEs are recognised as drivers of evolution, their activity is linked to various pathologies. Accordingly, TEs are under strict, layered regulation, which balances TE function with genome integrity. This is particularly important in early embryos and primordial germ cells (PGCs), to ensure the fitness of individuals but also their progeny. How do the different TE repressive pathways interact with each other? What role do they exert on cell identity? I hypothesise that the dynamic and context-specific repertoire of TE repressors is intimately connected to cell fate during embryonic and germ-cell development. To address this hypothesis, I will apply Perturb-seq (a screening method combining CRISPRi and scRNA-seq) in cultures of mouse embryonic stem cells and PGC-like cells to first, identify TE repressors and directly reveal their impact on cell fate through transcriptomic changes. Second, I will exploit Perturb-seq data to render a holistic view of TE repression programs, their interconnections, and their impact on cell fate. Finally, I will profile the binding of new TE repressors, describe the repression mechanisms, and understand their influence on the chromatin landscape governing cell fate changes. This project will identify how TE repression mechanisms influence cell fate and reveal the interplay between the different networks of TE repressors along with their own specificity towards different TE families. As inappropriate TE repression is an important component of cancer and infertility, this work will uncover new therapeutic approaches.

Poster N°11 : Molecular and cellular consequences of KIF14 methylation

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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.

Keywords: 5

Non-histone protein methylation, Kinesin, Cancer, Cytokinesis, Biochemistry

Poster N°12 : Identifying essential genes in host-parasite interactions using CRISPR technologies

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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 for pathogen 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.

Poster N°13 : Impact of pediatric high-grade glioma-associated histone H3 mutations on genome integrity

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Chromatin components, including histone proteins, contribute to preserve genome and epigenome integrity, thus protecting cells against tumorigenesis. Dominant mutations in H3 variants are observed in several types of cancers, among which pediatric high-grade glioma (pHGG), a currently incurable disease. These mutations promote oncogenesis by dysregulating gene expression through alterations of histone modifications. We identified an independent oncogenic mechanism in the two most frequent H3.3 mutations in pHGG, K27M and G34R. These mutations drive an aberrant non-homologous end joining (NHEJ) repair of replication-associated DNA damage, by perturbing the chromatin landscape and dysregulating the recruitment of repair factors to damaged replication forks, which leads to mitotic aberrations and fosters genome instability. In particular, we identified a DNA repair enzyme, Polynucleotide Kinase 3'-Phosphatase (PNKP), which sustains the proliferation of cells bearing H3.3 mutations, thus conferring a specific molecular vulnerability, with potential for therapeutic targeting. Our current work aims to characterize and validate this new therapeutic target in cellular and preclinical models. Furthermore, we are studying an additional mutation found in pHGGs, H3.1 K27M, to understand how K27M mutations on different H3 variants may similarly or differentially affect DNA repair capacities and thus the potential response of pHGGs to treatment.

Poster N°14 : H2Av participates in Transposable Elements silencing in *Drosophila* germline

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Transposable Elements (TEs) represent 15% of *D. melanogaster* genome and many families are still active. TEs expression and insertion is particularly deleterious in the germline since mutations are transmitted to the progeny and TEs over-expression often induces sterility in fruit flies. Fortunately, the genome has its own immune system based on small non-coding RNAs, which target TEs by sequence complementarity: piRNAs and siRNAs. Associated with Piwi family proteins, they inhibit TEs expression at the transcriptional or post-transcriptional level. Although the role of epigenetic marks such as H3K9me3 in TEs silencing and piRNAs expression has been extensively documented, **little is known about the use of histone variants to control TEs expression**.

Histone variants strongly impact nucleosome accessibility, playing important functions in eukaryotic genome regulation: transcription control, DNA repair, cell division, … Interestingly, while mammals have up to 10 H2A variants with different functions, *D. melanogaster* has only one, called H2Av. Hence, the single H2Av is required to cope with different functions, such as transcription regulation, to heterochromatin formation and to elicit DNA damage response by marking sites of DNA double-strand breaks upon phosphorylation.

In the lab, we have uncovered a role for H2Av in oogenesis, since its depletion leads to a developmental delay, oogenesis arrest and sterility. We show that this phenotype is due to the accumulation of replication stress and to the activation of DNA damage checkpoints proteins, such as chk2 and p53. Importantly, heterochromatin marks such as HP1□□and HP1□ (rhino) are affected. Hence, TEs are highly expressed in H2Av mutants and piRNA clusters are not expressed. To better understand the role of H2Av in heterochromatin formation during oogenesis, we are currently looking atH2Av genome-wide localization, especially at piRNAs clusters and TEs loci.

Altogether, we have evidences of a **new role of H2Av in the defense and maintenance of *D. melanogaster* genome integrity in the germline**, by controlling TEs expression.

Poster N°15 : Centromere structure as a novel pathway regulating immune cell aneuploidy associated with aging

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In the immune system, aging is characterized by gradual dysfunction and the increase of systemic inflammation, changes termed immunosenescence. The relationship between immunosenescence and cellular senescence is unclear. In blood, an age-associated feature of immune cells is the alteration of chromosome numbers (aneuploidy). Faithful chromosomal segregation is enforced by the stability and function of centromeres. Here, we investigated the regulation of centromere functionality in T lymphocytes and its implication for aneuploidy and cellular senescence. Centromeres are epigenetically defined by two core DNA-binding proteins, CENP-A and CENP-B. We have found that resting human lymphocytes from adults harbor a significant population of cells expressing low levels of total CENP-A, while CENP-B is not affected. Notably, CENP-A^{Low} cells show lower or non-detectable CENP-A loaded at centromeres, indicating a change in centromere identity. In contrast, T cells from newborns do not exhibit this population. Furthermore, CENP-A^{Low} T cells are more enriched in the naïve subset of adult T cells compared to memory populations. This indicates that the CENP-A-low state is associated with age-dependent changes and the functional state of T cells. *In vitro*, activated T cells in which we have recapitulated this defective centromere structure by genetic knock-out of CENP-A, show upregulated p53 target genes, proinflammatory genes and chromosome-specific aneuploidy, which are hallmarks of senescence. We have observed that CENP-A protein is downregulated only after DNA repair pathways are activated in X-irradiated resting CD4+ T lymphocytes, suggesting that there is an active degradation pathway activated downstream DNA damage. Overall, our results reveal that centromere structure is impacted through lifespan and upon DNA damage and determines aneuploidy in T cells, contributing to regulation of senescence and inflammation. Our work in progress focuses on the molecular mechanisms triggering centromere erosion in T lymphocytes and other quiescent human cells.

Poster N°16 : Methylator, a complete workflow for DNA methylation analysis

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DNA methylation is an epigenetic modification present in many organisms, including bacteria, plants, and mammals. Relatively stable across cell divisions, DNA methylation can be deposited or removed by enzymes and has been shown to regulate gene expression, genomic imprinting, X-chromosome inactivation, and repression of transposable elements. Analysis of epigenomes is fundamental to describe and understand how organisms with hundreds of differentiated cells arise from one fertilized egg. It also serves as a biomarker of cell identity, aging, and is broadly altered in cancer or in a group of rare congenital diseases (Mendelian disorders of the epigenetic machinery).

With short-read sequencing technologies, methods like Whole Genome Bisulfite Sequencing (WGBS) and RRBS (Reduced Representation of Bisulfite Sequencing) allow quantification of DNA methylation at the base level resolution to establish DNA methylation profiles of entire genomes. However, the analysis of these approaches requires large storage capacities, high computing resources, and utilizes many tools, and the field suffers from a lack of consensus workflow.

We aim to make these complex analyses achievable by biologists with little to no bioinformatics background, respecting the FAIR principles. To do so, we created Methylator, a complete Snakemake-based workflow for performing descriptive and differential DNA methylation analyses with robust and widely used tools. Cloned from our Unit's GitHub, Methylator runs from a dedicated Apptainer image containing all the required tools, apps, and R packages. It has been optimized to efficiently compute data on HPC clusters with a Slurm scheduler (IFB and iPOP-UP clusters).

Compared to existing workflows, Methylator is also a framework for benchmarking DNA methylation analysis tools and methods. The architecture allows for the addition of Snakemake rules to test or implement new tools or steps in our analysis. We added direct Nanopore sequencing analysis tools in parallel with short-read workflows, and we can easily compare WGBS, RRBS and Nanopore sequencing with or without adaptive sampling.

To run Methylator, you need two text files providing your "sample IDs" and analysis tool "parameters". Samples can come from GEO, FASTQ files, or BAM (Nanopore) files. An optional custom BED file can be provided for multiomic integrative analysis (e.g., with ChIP-seq or ATAC-Seq peaks). Methylator quantifies and annotates DNA methylation at the entire genome level, generates HTML reports for easy sharing with colaborators, bigwig files for visualization in IGV, and Rdata files for more personalized post-analyses and figure editing with R.

Poster N°17 : Identification of a pre-tumoral state characterized by partial EMT and activation of FGF signaling in breast epithelium

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Early detection of cancer is a determinant of patient outcome. Identifying markers and mechanisms of cell transformation remains a challenge to unlock novel precision medicine strategies, in particular for germline mutation carriers with enhanced predisposition to develop cancer. In the case of *BRCA1* mutation carriers, breast tumors have been proposed to originate from luminal progenitor cells. Yet how luminal cells transform into invasive cancer cells remains poorly understood. Here, we reconstitute sequences of events leading luminal cells to breast tumor formation combining single cell transcriptomics & epigenomics. Exposed to Brca1 and Tp53 loss, luminal progenitors can tolerate multiple genomic alterations without transforming, displaying an extensive epigenomic disorder. We further show that they progress to tumor formation through a partial epithelial-to-mesenchymal transition driven by Snail and a timely activation of autocrine and paracrine FGF signaling. Tumor initiation is delayed in mice treated with an FGFR inhibitor, proposing FGF signaling as a vulnerability of these pre-tumoral cells. In humans, such pre-tumoral events are detected in early stage basal-like tumors that rarely recur, and normal-like mammary glands of *BRCA1* mutation carriers. Our study bridges gaps of *BRCA1*-tumorigenesis, opening perspectives for the monitoring of *BRCA1* carriers with high tumor initiation probability.

Poster N°18 : Characterizing stress response variability in oligodendrocyte lineage upon neuroinflammation at single-cell resolution

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Neuroinflammation in premature infants is often accompanied by diffuse white matter injury (DWMI) caused by the blockade of **oligodendrocyte precursor cells** (OPC) differentiation and maturation. Not all OPCs are equally affected by stress allowing the brain to fully develop, however cortical **hypomyelination** increases the risk of neurological diseases.

In order to better characterize the inflammatory response of the oligodendrocyte cell lineage in the early mouse brain pup, we generated **single cell multiomics data,** from oligodendrocyte lineage sorted cells, using a perinatal inflammatory mouse model.

The analysis of such multiomics data, combining transcriptomic and chromatin accessibility information from the same cell**,** among oligodendrocytes-enriched cell populations, allowed us to identify the cells that are the **most sensitive to stress** and hypothesize which **molecular pathways** could be implicated.

Our data allow us to refine the knowledge on the oligodendrocyte maturation trajectory and to show that mainly immature cells from the OL-lineage were altered by stress compared to already committed cells. We also show that inflammatory response pathways are predominantly up regulated in these vulnerable cell populations.

Poster N°19 : Deciphering DNA methylation maintenance mechanisms is response to DNA Damage

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DNA methylation is a key epigenetic mark that drives mammalian development and transcriptional states. Many human diseases including several cancers are characterized by an altered methylome. It is therefore crucial that DNA methylation marks are faithfully re-established not only during DNA replication but also after DNA damage and repair. However, whether and how DNA methylation is maintained at sites of DNA damage is still poorly understood.

To address this question, I focus on DNA damage caused by UV irradiation in mammalian cells. Through imaging approaches, I showed that most DNA methylation marks were maintained after UV damage repair and I characterized the molecular machinery involved. I detected the recruitment of DNMT1 and DNMT3A, two DNA methylating enzymes, to sites of UV repair, as well as the DNMT1 co-factor UHRF1. I also dissected their functional interactions with the UV damage repair machinery and repair-coupled histone dynamics. I am also developing a live imaging approach based on UVC-laser irradiation of methylation-enriched heterochromatin domains, to study DNA methylation dynamics after DNA damage in real time and a Nanopore-based sequencing approach to refine the mapping of DNA methylation marks at sites of DNA repair. Finally, I aim at characterizing the functional relevance of DNA methylation maintenance at UV sites.

Overall, this study should lead to a detailed picture of DNA methylation dynamics at sites of repair synthesis, with important implications for understanding DNA methylation alterations in human diseases.

Poster N°20 : X-chromosome inactivation is essential for human female extraembryonic cell specification.

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X-chromosome inactivation (XCI) is a developmentally regulated process taking place in female mammals, where one of the X chromosomes is silenced. The non-coding RNA XIST acts as a key regulator of XCI in mice, and its absence disrupts the initiation of XCI. Proper XCI is essential for the growth of extraembryonic tissues during female mouse development. However, species specificities in mechanisms and timing leading to XCI during early embryogenesis leave open the key question of the interdependence between XCI and human development. To study these questions, we leveraged the capacity of human naïve pluripotent stem cells (PSCs) to differentiate into extraembryonic mesoderm cells (EXMCs) and trophoblast stem cells (TSCs), two crucial post-implantation extraembryonic tissues. We show that the differentiation of human pluripotent stem cells to trophoblast stem cells and extraembryonic mesoderm cells triggers XCI. Using CUT&RUN, we find that the inactive X chromosome in extraembryonic cell lineages displays an atypical chromatin state, not associated with classical heterochromatin markers. We further demonstrate that extraembryonic specification and XCI are kinetically and functionally linked. Using loss-of-function approaches, we formally prove for the first time that XIST is required for human XCI establishment. In addition, absence of XIST, and thus XCI, leads to cell death during extraembryonic cell specification. Single-cell RNA sequencing at various time points reveals that cells can exit the naïve pluripotency state in absence of XCI and undergo transcriptional changes characteristic of EXMC/TSC cell fate acquisition, but that their survival is compromised.

Our work therefore links X-chromosome dosage to the formation of extraembryonic annexes, with potentially important consequences for human reproductive biology.

Poster N°21 : Proteomic profiling of UV damage repair patches uncovers histone chaperones with central functions in chromatin repair

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DNA damage challenges both genome and epigenome integrity, and thus jeopardizes the maintenance of cell identity. Indeed, DNA damage repair is accompanied by a re-organization of chromatin. In response to UV lesions in human cells, it involves the redistribution of parental histones and the incorporation of newly synthesized histones. This leads to changes in histone variants and histone post-translational modifications, and therefore modifies the epigenetic landscape at repair sites.

To elucidate the mechanisms involved in epigenome maintenance at DNA repair sites in human cells, we developed an unbiased proteomic approach based on Isolation of Protein On Nascent DNA at Repair sites (IPOND-R) combined with mass spectrometry. Among the proteins enriched at UV-induced damage sites, we identified DNAJC9 and MCM2, two histone chaperones that had not been previously associated with the UV response. We studied their implication in histone dynamics at repair sites. We found that DNAJC9 and MCM2 cooperate to promote new H3-H4 deposition, by supplying the histone chaperones CAF-1 and HIRA, and to promote also parental H3-H4 recovery at DNA repair sites. Overall, this study provides a better understanding of the mechanisms controlling histone dynamics in response to UV-induced damage, and puts forward IPOND-R as a powerful methodology to unravel new epigenome maintenance mechanisms.

Poster N°22 : Epigenetic and Epitrancriptomic regulations during development and posterior regeneration in the annelid *Platynereis dumerilii*.

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Changes in cell identity, which are essential features of embryogenesis and regeneration, require massive variation in gene expression. Epigenetic marks, such as m5C DNA methylation, are well described in mammal models. They are known for their role in modulating gene transcription and in the formation/maintenance of different cell lineages. Upon transcription, mRNAs can also undergo chemical modifications impacting their splicing, export stability or translation, thus adding an epitranscriptomic layer to gene regulation. One of the most common mRNA modications, m6A, is well described in mammalian development. However, little is known about the importance of these two types of methylation in other animals, and other developmental contexts.

The marine annelid *Platynereis dumerilii* is a suitable non-mammalian model to study the role of both m5C and m6A methylation during developmental processes. We are studying two of those processes: embryonic development and regeneration of the posterior part after amputation. Our aim is to determine the dynamics of these methylations, the consequences of altering their machineries and whether there is a direct connection between them.

Previous work in our lab showed a high level and a dynamic expression of the m5C methylation and its machinery, throughout *Platynereis dumerilii*'s lifecycle. Furthermore, treatments using decitabine (a hypomethylating agent) deteriorate both development and posterior regeneration (Planques et al. 2021). We are extending our investigation using Whole Genome Bisulfide Sequencing to describe m5C dynamics. Concerning m6A RNA methylation, we identified most of the epitranscriptomic machinery (writers, erasers, readers) in *Platynereis dumerilii*. Furthermore, mass spectrometry results and our transcriptomic data have respectively revealed a dynamic expression of both m6A methylation, and genes involved in its machinery, throughout *Platynereis dumerilii*'s life cycle. Functional analyses have been initiated thanks to the use of STM2457, a chemical inhibitor of METTL3. They have shown that m6A impairment induces a slowdown of the entire regenerative process. We are currently delving deeper into this phenomenon, at the molecular level, employing a panel of *in situ* hybridization markers and labelling techniques such as TUNEL for cell death or EdU for S-phase entry. Moreover, with the recent implementation of CRISPR/Cas9 technology into our laboratory, we are ready to conduct knock-out experiments to elucidate the role of this methylation. To this end, we have chosen the reader FXR1 homologs as first candidate genes for its potential role in directly linking regulations through m6A and m5C methylations (Deng et al. 2022).

Poster N°23 : Regulation of X chromosome inactivation across cell lineages and impact on cell function

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In mammals, males (XY) and females (XX) do not exhibit the same susceptibilities to the development of certain diseases, particularly those affecting the neural system. These differences are not solely explained by distinct hormonal contexts but may also be related to the regulation of the expression of sex chromosomes. The inactivation of the X chromosome (XCI), which equalizes X-linked gene expression between XX and XY cells, early in the embryogenesis, by globally repressing one X chromosome, constitutes a major regulation of X-linked gene expression. However, in the hematopoietic lineage a tolerated plasticity of XCI is observed leading to a partial derepression of certain Xi genes. Additionally, in all adult tissues, certain genes escape XCI and are also expressed from the Xi. The identity of these genes and their level of escape from XCI vary depending on cell types and differentiation stages, indicating that the Xi could undergo customized remodeling of its expression profile according to cellular contexts, thus conferring specific functionalities to female cells.

In this context, my project aims to 1) determine whether XCI plasticity exists during human neural differentiation, and 2) disrupt XCI by inducing the loss of XIST at different stages and study its effect on cellular homeostasis. To do this, I will differentiate human embryonic stem cells (hESC) into neural cells and study various aspects of X chromosome regulation along the process. Characterization of Xi remodelling will include single-cell imaging of 3D organization, analyses of Xi chromatin composition using CUT1RUN, RNA antisense purification (RAPseq) and transcription profiling using RNAseq. I will also use a 3D model, human Cortical Organoid (hCO) to assess the inactive X in different neural cell type. To address how XCI regulation affects cellular function, I will perturb the expression of XIST – the major actor of XCI – using genetically-engineered hESC lines.

This project should bring critical information on the dynamics of XCI regulation in response to cell lineage commitment and on the role of XIST in the maintenance of XCI thereby leading to a better understanding of male/female differences in pathological contexts.

Poster N°24 : Understanding the chromatin-mediated regulation of PTB-dependent splicing via proximity labeling

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Alternative splicing is an essential process for protein diversity and cellular identity, and its deregulation can lead to cancer. Recently, we discovered a role for histone marks in dynamically regulating splicing during epithelial-to-mesenchymal transition (EMT) [1]. It has been shown that the H3K27ac mark enables the recruitment of an essential splicing factor, PTBP1, thereby altering splicing at this specific locus. Genome-wide ChIP-seq and RNA-seq studies have shown that among PTB-dependent splicing events changing during EMT, 13% also show changes in the level of H3K27ac, compared with only 4% of H3K4me3.

To verify this proximity, I performed high-resolution IFs and PLAs between PTBP1 and H3K27ac, or between PTBP1 and H3K4me3. Finally, to find the mechanistic link between H3K27ac and PTBP1, I'm using an innovative proximity labeling technique based on the use of an antibody. After targeting an APEX biotin ligase to PTBP1 via this antibody, I'll be able to identify all nearby proteins. Coupling this with cell fractionation to isolate the chromatin bubble, as well as proximity labeling on H3K27ac, and crossing them with genome-wide data, I will be able to understand the role of this histone mark and its regulatory proteins in PTBP1-dependent splicing during EMT.

[1] Segelle et al., Cell Rep 2022

Poster N°25 : DNA methylation shapes the Polycomb landscape during the exit from naïve pluripotency

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In mammals, 5 methyl-cytosine (5mC) and Polycomb Repressive Complex 2 (PRC2)-deposited histone 3 lysine 27 trimethylation (H3K27me3) are generally mutually exclusive at CpG-rich regions. As mouse embryonic stem cells exit the naïve pluripotent state, there is massive gain of 5mC concomitantly with restriction of broad H3K27me3 to 5mC-free, CpG-rich regions. To formally assess how 5mC shapes the H3K27me3 landscape, we profiled the epigenome of naïve and differentiated cells in the presence and absence of the DNA methylation machinery. Surprisingly, we found that 5mC accumulation is not required to restrict most H3K27me3 domains. Instead, this 5mC-independent H3K27me3 restriction is mediated by aberrant expression of the PRC2 antagonist *Ezhip*. At the subset of regions where 5mC appears to genuinely supplant H3K27me3, we identified 163 candidate genes that appeared to require 5mC deposition and/or H3K27me3 depletion for their activation in differentiated cells. Employing site-directed epigenome editing to directly modulate 5mC levels, we demonstrated that 5mC deposition is sufficient to antagonize H3K27me3 deposition and confer gene activation at individual candidates. Altogether, we systematically measured the antagonistic interplay between 5mC and H3K27me3 in a system that recapitulates early embryonic dynamics. Our results suggest that H3K27me3 restraint depends on 5mC, both directly and indirectly. Our study also implies a non-canonical role of 5mC in gene activation, which may be important not only for normal development but also for cancer progression, as oncogenic cells frequently exhibit dynamic replacement of 5mC for H3K27me3 and vice versa.

Poster N°26 : Predictive modelling of Acute Promyelocytic Leukaemia resistance to Retinoic Acid therapy

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Acute Promyelocytic Leukaemia (APL) results from an aberrant chromosomal translocation involving the Retinoic Acid Receptor Alpha (RARA) gene, predominantly with the Promyelocytic Leukaemia (*PML*) or Promyelocytic Leukaemia Zinc Finger (*PLZF*) gene. These oncoproteins block the haematopoietic differentiation program promoting aberrant proliferative promyelocytes. Retinoic Acid (RA) therapy is successful in most of the PML::RARA patients, while PLZF::RARA patients frequently become resistant and relapse. Recent studies pointed to various underlying molecular components, but their precise contributions remain to be deciphered.

We developed a logical network model integrating signalling, transcriptional and epigenetic regulatory mechanisms, which captures key features of the APL cell responses to RA depending on the genetic background. The explicit inclusion of the histone methyltransferase EZH2 allowed the assessment of its role in the resistance mechanism, distinguishing between its canonical and non-canonical activities.

The model dynamics was thoroughly analysed using tools integrated in the public software suite maintained by the CoLoMoTo consortium (https://colomoto.github.io/).

The model serves as a solid basis to assess the roles of novel regulatory mechanisms, as well as to explore novel therapeutical approaches *in silico.*

Poster N°27 : Investigate HSF2 contribution to human neural development.

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Neurodevelopmental disorders (NDDs) encompass a heterogeneous group of disorders that result from defective brain development and affect ~10% of children, causing lifelong handicap. Their diversity and inaccessibility of the brain tissue have been a brake for understanding the basic mechanisms underlying the pathology and designing therapeutic strategies.

Regardless of their genetic or environmental origin, NDDs share common features, globally connected to Heat shock factors (HSFs). HSFs are stress-sensitive transcription factors and integrators of cell stress, development and lifespan, performing a broad spectrum of functions under both physiological and pathological conditions. Dysregulation of the HSF pathway is found in several pathological conditions (e.g cancer, inflammation, neurodegeneration) and aging. Among HSFs, HSF2 is spontaneously active during brain formation and plays a major role at multiple steps of prenatal cortex development, by regulating a large repertoire of neurodevelopmental genes. Either reduced or exacerbated HSF2 activity is associated to NDD of environmental (e.g. Fetal Alcohol Syndrome) or genetic origin (e.g. Rubinstein-Taybi syndrome (RSTS) and Angelman-like Syndrome (AGS-like)). Our lab has recently shown that the CBP/EP300 acetyltransferases, mutated in RSTS, mediate HSF2 acetylation and protein stabilization; indeed, HSF2 protein levels and activity are defective in RSTS fibroblast.

Here, we investigate HSF2 contribution to early stages of neurodevelopment, focusing on RSTS as a paradigm model of HSF pathway dysregulation. To this aim, RSTS patient cells are reprogrammed to hiPSCs and used to generate cerebral organoids (hCOs). In RSTS-hCOs, we observe a reduction in HSF2 protein levels, concurrent with dysregulation in HSPs and N-cadherin, crucial proteins involved in stress response and cell adhesion. Additionally, the normal positioning of mitoses at the neuroepithelium apical surface is disrupted in RSTS hCOs. Moreover, premature neuronal differentiation is evident in RSTS hCOs compared to healthy donors (HD), as indicated by the early cortical neuron marker TBR1 expression.

To comprehensively understand the impact of HSF dysregulation on neural differentiation we used two complementary approaches. First, to identify in an unbiased way HSF2-dependent pathways, we conducted a multi-ome single-cell analysis on hCOs derived from RSTS and HD hiPSCs at two differentiation stages (DIV25 and DIV45). Although our analysis pipeline is still being optimized, initial findings validate dysregulations in the HSPs and cadherin superfamily. In parallel, we developed a "rescue" approach by restoring HSF2 protein levels in RSTS. This was achieved through HSF2 editing at the genomic locus via CRISPR/Cas12. Preliminary results suggest that recovery of HSF2 protein levels is sufficient to ameliorate some of the observed defects in RSTS hCOs.

Globally, our work contributes to uncover the potential of HSF2 to ensure proper human neurodevelopment, and how its dysregulation can drive pathological traits.

Poster N°28 : The Lonely Pipette podcast; helping scientists do better science

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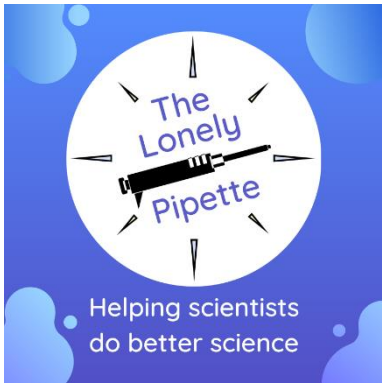
The Lonely Pipette is a month podcast presenting long-format discussions with scientists around the world. The hosts are Jonathan, a professor of genetics and epigenetics and team leader, and Renaud, an independent science communicator. Renaud and Jonathan talk to inspiring people to learn about their 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!



https://thelonelypipette.buzzsprout.com/

bit.ly/TLPtree

Poster N°29 : Impact of ZBTB38 on Chromocenter Stability: Linking DNA Methylation to Chromosomal Breakage

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Proper cellular function depends on a carefully choreographed pattern of gene expression, and alterations of this process are linked to a plethora of human diseases (Chatterjee and Ahituv, 2017; Rickels and Shilatifard, 2018). This gene expression pattern is especially important during embryonic development, where gene expression is very dynamic and needs to be tightly regulated for each cell to differentiate and form new tissues. The regulation of gene expression is influenced by external cues and determined by the interaction of the transcriptional machinery with genetically encoded regulatory elements and with the cell's specific epigenetic landscape (ENCODE Project Consortium et al., 2020). Three key epigenetic determinants are chromatin accessibility, local histone post-translation modifications, and DNA modifications, mainly in the form of cytosine methylation (5mC) and its derivatives.

In mammals, DNA methylation is predominantly cytosine methylation at the C5 position, yielding 5-methylcytosine (5mC) mostly in the context of CpG dinucleotides (Li and Zhang, 2014). The pattern of genomic CpG methylation is not random: it is stable within a cell type and presents reproducible differences between varying cell types (Schultz et al., 2015). It has been established that DNA methylation at promoters and gene bodies has a causal role in the regulation of gene expression (Luo et al., 2018). This applies to single-copy genes, but also to the many transposable elements that populate mammalian genomes (Smith and Meissner, 2013). DNA methylation is interpreted by various regulators, including methyl-CpG-binding domain (MBD) proteins, which can repress transcription by disrupting transcription factor binding and recruiting co-repressors to create a repressed chromatin environment.

Although key molecular players have been identified, the process is not fully understood. The complete set of factors that bind methylated regions and repress transcription remains to be identified. Forty-nine genes in the human genome encode Zinc finger and BTB (ZBTB) proteins (Siggs O & Beutler B, 2012), a family of proteins that contain N-terminal BTB (Broad complex, Tramtrack and Bric-à-brac) domains and different numbers of C-terminal ZF (Zinc Fingers) motifs. Out of these 49 proteins, 3 of them (ZBTB33/KAISO, ZBT38/CIBZ and ZBTB4) were shown exhibit bimodal DNA recognition within their homologous three C_2H_2 ZF domains and to bind methylated DNA *in vitro* (Daniel et al., 2002 and Fillion et al., 2006). Here, we aim to investigate the role of ZBTB38 in the repression of gene expression and genome integrity maintenance in mouse embryonic stem cells (mESCs) and most especially, their role at methylated regions of the genome.

Poster N°30 : EDC platform consortium

Research at EDC lab is facilitated on a daily basis by a set of technical platforms and support services. In April 2021, the six EDC platforms have created a consortium to increase the interactions between the platforms, as well as their visibility. In October 2021, during the lab retreat, half a day was dedicated to a platform workshop where everyone in the lab got the opportunity to get to know all the platform activities and to make suggestions for future developments. Since then, joint or specific seminars are given by all platform managers to give answers to the lab demands and expose their road map. The consortium reinforced existing interactions, such as the ones between the GENIE and the Vectorology platforms or between EpHISTain and EPI2, and allowed new interactions to emerge, for instance for the development of Nanopore sequencing, where the EpiG and BiBs platforms have team up to offer lab users the possibility to sequence and analyse their data in house.
The poster will present the 6 platforms as well as the ddPCR extra service. It will also present the associated platforms for bioinformatics at UPCité (iPOP-UP), for organoids production (enSCORE) and for single-cell sequencing (WISCI).

Authors:
- Christophe Huret (Vectorology)
- Laure Ferry (EpiG, Functional Epigenomics)
- Magali Hennion (BiBs, Bioinformatics and Biostatistics)
- Olivier Kirsh (BiBs, Bioinformatics and Biostatistics)
- Ekaterina Boyarchuk (GENIE, Genome Engineering in Epigenetics)
- Sandra Piquet (EPI2, Epifluorescence and confocal microscopie for Epigenetics)
- Audrey Chansard (EPI2,Epifluorescence and confocal microscopie for Epigenetics)
- Myriam Mohamed (EpHISTain, Histology and Cytology)
- Laurence Del Maestro (ddPCR, Droplet Digital PCR)
- Bertrand Cosson (iPOP-UP, integrative Platform for Omics Projects at UP, multisite)
- Sandrine Adiba (enSCORE, engineered Spinal and Cortical Organoid coRE, mutualized with IJM)
- Kamal Bouhali (enSCORE, engineered Spinal and Cortical Organoid coRE, mutualized with IJM)
- Mikaëlle Bocel (enSCORE, engineered Spinal and Cortical Organoid coRE, mutualized with IJM)
- Brianna Rodgers (WISCI, Who am I Single Cell Initiative, multisite)