LIFESCIENCE PHD SYMPOSIUM

LifeScience PhD Symposium of the Institute of Genetics and Development of Rennes

4th to 5th November 2024

Le Diapason
Université de Rennes - Campus de Beaulieu
35700 Rennes
France

Program

Time	Event	Location	Speaker
Day 1			
13:00–13:40	Registration and welcoming with coffee, tea and juices		
13:45–14:00	Presentation of the event	Amphitheatre	Reynald Gillet (Director of IGDR) and Vasantha (Organizing Team Member)
14:00–15:30	**Research to Better Understand Diseases and Propose New Therapies**		
14:00–14:45	Multi-modal Learning for Single-cell Data Integration	Amphitheatre	Laura Cantini (Keynote Lecture)
14:45–15:00	LightSpot®-FL-1, an Innovative Tool for the Detection and Quantification of P-gp, a Predictive Biomarker of Tumor Resistance in Acute Leukemia Models	Amphitheatre	Maxime Dubois
15:00–15:15	Autism-related Gene Intergenerationally Regulates Neurodevelopment and Behavior in Fish through Non-genetic Mechanisms	Amphitheatre	Constance Merdrignac
15:15–15:30	RBM22-depletion Delays Progression through All Steps of Cell Cycle and Increases Ploidy in Myeloid Cells	Amphitheatre	Eloïse Le Hir-Reynaud
15:30–16:15	Flash Talks	Amphitheatre	Young Researchers
16:15–16:30	Sponsor Presentation by Merck	Amphitheatre	Merck
16:30–18:30	Poster Session I	Poster Exposition Space	
19:00–21:30	Social Event and Gala Dinner		

Time	Event	Location	Speaker
Day 2			
08:15–09:00	Welcoming with Breakfast		
09:00–10:45	**In Silico Biology**		
09:00–09:45	Synthetic DNA as the Future of Data Archiving—Technological Advances and Challenges	Amphitheatre	Marc Antonini (Keynote Lecture)
09:45–10:00	ANNEXA: A Comprehensive Pipeline for Extending Genome Annotations Using Long-Read Transcriptome Sequencing	Amphitheatre	Nicolaï Hoffmann
10:00–10:15	Role of Circular RNAs in Treatment Resistance of Cutaneous Melanoma	Amphitheatre	Rose-Marie Fraboulet
10:15–10:30	Structural Study of the Hibernating Ribosomes in Pathogenic Bacteria	Amphitheatre	Vasanthakrishnan Radhakrishnan Balasubramaniam
10:30–10:45	Super-resolution Radial Fluctuations to Investigate the Mitochondrial Morphology in AURKA Over-expression	Amphitheatre	Nicolas Jolivet
10:45–12:45	Poster Session II	Poster Exposition Space	
13:00–13:55	Lunch Break		
14:00–15:45	**From "*in vivo*" to "*in vitro*"**		
14:00–14:45	From Molecules to Ecosystems: Microbial Predators in Soil Ecology	Amphitheatre	Tâm Mignot (Keynote Lecture)
14:45–15:00	Structure and Function of Spermatid Manchette Microtubules	Amphitheatre	Céline Callens
15:00–15:15	The Transcriptional Dynamics of Muscle Progenitors Response to Notch Signaling	Amphitheatre	Emma Leroux
15:15–15:30	Molecular Mechanisms of Aneuploidy and Glioblastoma Aggressiveness Induced by Diaph3 Loss	Amphitheatre	Caren Jabbour
15:30–15:45	Kinetochore Microtubules Flux Poleward along Fixed Centrosome Anchored Microtubules during the Metaphase of *C. elegans* One-cell Embryo	Amphitheatre	Mathis Da Silvas
15:45–16:00	Award Ceremony		
16:00–16:15	Symposium Closing by the Organizing Committee		

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Rennes Métropole

Rennes Métropole is the intercommunal structure centred on the city of Rennes. It is located in the Ille-et-Vilaine department, in the Brittany region, western France. It aims to build a better metropolitan area by synchronizing the transport system, environmental actions, urbanization, economic and social development, culture, university research, etc.

La Ligue Contre le Cancer

"La Ligue Nationale contre le Cancer" (French League against cancer) is a public interest association founded in 1918 after WWI, when cancer was recognized as a spreading epidemic. The League's goal is to help cancer patients, their family and friends. Since its founding, the League has developed into a strong network and leads the fight against cancer on three levels: research, promotion of screening and prevention, and care for patients and their loved ones. The League is a federation of 103 departmental committees that are active in relaying the mission of the administrative council and the national scientific council. The League has more than 720,000 members, 30,000 volunteers and 300 salaried staff.

IGDR

At the IGDR, our primary focus is on cell and developmental biology, and genetics. We emphasize two main scientific axes: Genomic Biology and Cancer (GBC) with 6 teams, and Cell Biology, Cell Development, Biophysics (CCB) with 9 teams. These axes are interconnected, reflecting our commitment to understanding life's dynamics. We've established "Observing Life" as a third transversal theme to integrate these areas. The GBC teams study genetic materials and alterations, primarily through nucleic acid analysis and (deoxy)ribonucleoprotein complex studies. The CCB teams focus on observing cellular and multicellular organisms.

University of Rennes

The University of Rennes is an experimental public institution. Open to Europe and the world, at the heart of the Brittany Region and linked to Rennes Métropole and its ecosystem, it is built on a common history and the strengths of its founding members. It has one ambition: to meet the major societal challenges of a world in transition, particularly in the fields of the environment, global health, and digital technology.

CNRS

The National Centre for Scientific Research (CNRS) is a major player in basic research on the global stage and the only organisation in France that is active in all fields of science. Its unique position as a specialist in multiple fields means that it can bring together different scientific disciplines to shed light on and gain insight into current global challenges in partnership with public sector, social, and economic stakeholders.

INSERM

Founded in 1964, Inserm is a public scientific and technological institute which operates under the joint authority of the French Ministries of Health and Research. The institute is dedicated to biomedical research and human health and is involved in the entire range of activities from the laboratory to the patient's bedside. It also partners with prestigious research institutions committed to scientific challenges and progress in these fields.

PhD Symposium

Sessions

Research to better understand diseases and propose new therapies

Multi-modal learning for single-cell data integration

Laura Cantini *† [1]

[1] UMR3738 : Developmental and Stem Cell Biology, Institut Pasteur – France

Laura Cantini is a G5 junior group leader heading the Machine Learning for Integrative Genomics group. She is a CNRS researcher and a Prairie chair.

Mathematician by training, Laura Cantini works at the interface of machine learning and genomics. Due to the advent of high-throughput technologies, multiple large-scale quantitative measurements, a.k.a. omics, can be accessed for the same set of biological samples or cells. The focus of Laura Cantini's research activity is to design machine learning methods able to co-analyze the numerous available omics data and translate them into actionable biological knowledge. By applying the developed approaches to patient-derived data, she contributes to improve our understanding of cancer heterogeneity and its underlying molecular mechanisms.

Laura Cantini received several awards: CNRS Bronze Medal (2024), Prix Paoletti (2022), L'Oréal-UNESCO for Women in Science (2018).

Keywords: Multi-modal, Single-cell

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Research to better understand diseases and propose new therapies

Short-talks

LightSpot®-FL-1: An Innovative Tool for Detection and Quantification of Permeability-Glycoprotein (P-gp), a Predictive Biomarker of Tumor Resistance in Acute Leukemia Models

Maxime Dubois * [1], Antoine Goisnard * [1], Céline Bourgne * [2], Elodie Gay * [1], Marie Depresle * [1], Manon Roux * [1], Allison Voisin * [2], Marc Berger * [2], Pierre Daumar * [1], Emmanuelle Mounetou * [1], Mahchid Bamdad† [1]

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[2] *Role of Intra-Clonal Heterogeneity and Leukemic Environment in Therapy Resistance of Chronic Leukemias – EA 7453 CHELTER, CHU Clermont-Ferrand, Hôpital Estaing – France*

Permeability-glycoprotein (P-gp) is a xenobiotic efflux pump located on the cell membrane, playing a critical role in cellular resistance mechanisms by recognizing and expelling a diverse array of molecules, including anticancer agents. This activity significantly contributes to Multi-Drug Resistance (MDR) (1, 2). Assessing P-gp expression levels is essential in clinical diagnostics to prevent tumor resistance. However, no standardized method currently exists for detecting and quantifying P-gp in clinical practice. To address this, our research group has developed an innovative fluorescent conjugate, LightSpot®-FL-1, which specifically targets P-gp, allowing detection by flow cytometry or fluorescence imaging techniques (3, 4). This study evaluated LightSpot®-FL-1 efficacy in detecting P-gp in poor-prognosis acute leukemia cell lines (e.g., CCRF-CEM and KG-1a models). First, a P-gp labeling protocol using LightSpot®-FL-1 revealed differential P-gp expression between cell lines. Analysis showed that P-gp overexpression correlates with increased resistance to standard treatments in acute leukemia. This methodology will next be applied to blood cells from patients with acute leukemia, enabling identification of cancerous subpopulations with P-gp overexpression and potentially responsible for tumor resistance. In conclusion, this research may open new avenues for clinical diagnostics through the use of the innovative LightSpot®-FL-1 conjugate in cancer cell resistance detection.

References:

1. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta. 1976;455(1):152-62.

2. Bosch I, Croop J. P-glycoprotein multidrug resistance and cancer. Biochim Biophys Acta. 1996;1288(2):F37-54.

3. Daumar P, et al. Chemical biology fluorescent tools for*in vitro*investigation of P-gp expression in tumor cells. RSC Adv. 2023;13(39):27016-35.

4. Goisnard A, et al. LightSpot®-FL-1 Fluorescent Probe: Tool for Cancer Drug Resistance by Direct Detection and Quantification of P-gp. Cancers. 2021;13(16):4050.

Keywords: Acute Leukemia, Multi-Drug Resistance, P-gp Quantification, LightSpot®-FL-1 Tracers, Flow Cytometry

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Autism-related gene intergenerationally regulates neurodevelopment and behavior in fish through non-genetic mechanisms

Constance Merdrignac *† [1], Antoine Clement [1], Sergi Roig Puiggros [2], Aurélien Brionne [1], Thaovi Nguyen [1], Jérôme Montfort [1], Cervin Guyomar [3], Denis Jabaudon [4], Violaine Colson [5,6], Florent Murat [1], Julien Bobe [1]

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Parental experience can influence progeny neurodevelopment and long-term behavior through non-genetic modifications of the gamete (i.e., modifications that do not involve changes in inherited DNA sequence). Unlike paternal effects, maternal non-genetic mechanisms shaping long-term progeny neurodevelopment and behavior remain poorly understood in vertebrates. We used the medaka, a model fish species, to investigate the role of auts2a (the ortholog of human AUTS2), a gene repressed in the fish oocyte following maternal stress and associated with neurodevelopmental disorders (NDDs). Here we show that maternal, but not paternal, auts2a modulates long-term progeny behavior, including anxiety-like behavior and environment recognition capabilities. Using single-nuclei RNA-sequencing, we reveal that maternal auts2a modulates progeny early neurodevelopment by regulating gene expression, including in all neural cell populations. Among these regulated genes, a significant number have been identified as transcription factors, are parts of enriched pathways of interest (i.e., axon guidance, Wnt signaling pathway and/or regulating stem cell pluripotency), and/or have been associated with relevant NDDs. We show that maternal auts2a regulates maternally-inherited factors abundancy, including transcriptional and post-transcriptional regulators required for early development and zygotic genome activation. Together, our results reveal the unsuspected role of an oocyte-expressed NDD gene in shaping progeny neurodevelopment and long-term behavior in a vertebrate species. Finally, we report that auts2a/AUTS2 belongs to a group of evolutionarily-conserved oocyte-expressed genes responsible for NDDs. Our results raise the question of the maternal role of these oocyte-expressed NDD genes in the non-genetic control of progeny neurodevelopment and behavior in vertebrates.

Keywords: Autism spectrum disorders, Intergenerational effect, Germ cell drivers

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RBM22-Depletion Delays Progression Through All Steps Of Cell Cycle And Increases Ploidy In Myeloid Cells

Eloïse Le Hir-Reynaud *† [1], Benoît Soubise [1], Abrahan Molina-Mendoza [1], Cassandra Konan [1], Séverine Commet [1,2], Nadia Gueganic [1,2], Corinne Tous [1,2], Laurent Corcos [1], Nathalie Douet-Guilbert [1,2], Marie-Bérengère Troadec [1,2]

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RNA-Binding Motif 22 (*RBM22*) is a splicing factor gene, which is also a transcription regulator. *RBM22* protein also plays a potent role in cancers. Its role as splicing factor has been well studied whereas its function in cell cycle has not been much documented. The expression of *RBM22* can be altered in some cancers and phenotypic effects of *RBM22* depletion in hematological cells has been previously described, so we chose to work on a hematological model. The goal of our study was to better characterize the implication of *RBM22* in cell cycle progression, because very few data were available on this particular function of *RBM22.* *RBM22* haploinsufficiency (corresponding to the loss of one allele) has been observed in myelodysplastic neoplasms (MDS) with del(5q) because this gene is located on the commonly deleted region of the long arm of chromosome 5. Therefore, we focused on the impact of *RBM22* depletion on cell proliferation in MDS with del(5q) because MDS patients suffer from cytopenia. In this study, we showed that the depletion of *RBM22* reduces cell proliferation of myeloid cells. Surprisingly, *RBM22* depletion alters every step of cell cycle since its depletion delays the progression of the G1-phase, S-phase and G2/M phase. *RBM22*-haploinsufficiency also induces a mitosis defect, including endomitosis and an alteration of megakaryocyte differentiation of the MDS-L cell line. Here, we show an involvement of *RBM22* in cell cycle regulation, which could explain the phenotype of some cancers in which one allele is lost, as is the case in del(5q) MDS.

Keywords: RBM22, Cell cycle, del(5q) MDS

*Speaker
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Research to better understand diseases and propose new therapies

Posters and Flash-talks

Research to better understand diseases and propose new therapies

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Poster and flash-talk #15

Spatial transcriptomics and transdifferentiation in breast cancer tumors

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Triple negative breast cancer (TNBC) is a breast cancer subtype characterized by the absence of alterations in estrogen (ER) and progesterone (PR) receptors, as well as human epidermal growth factor receptor 2 (Her2). It is both a rare and aggressive subtype, representing between 10 and 20% of all breast cancer cases, and is unfortunately still not well characterized yet(3). To date, no biomarkers driving the very specific carcinogenesis mechanism of the TNBC tumors have been identified (1). As a matter of fact, TNBC is ineligible to targeted therapies, making it the most lethal breast cancer subtype.

TNBC subtypes such as metaplastic breast carcinoma (MpBC) are associated with high non-genetic plasticity responsible for high resistance to treatments. MpBC is characterized by an abnormal transdifferentiation of epithelial cells to a different phenotype, whose presence is not expected in the tissue, detected by histopathological review of tumor samples. Different types of MpBC exist, according to the trans-differentiated phenotype observed. The molecular mechanisms behind this plasticity remain however unknown. As different subtypes of MpBC display different survival rates, appropriate and specific molecular markers to complement pathology-based diagnostic options are also still required.

This study is aimed to provide new insights on the transdifferentiation of metaplastic tumors, with a specific focus on mesenchymal spindle cell tumor cells. The primary goal is to analyze the genomic, transcriptomic as well as the microenvironmental differences between the different trans-differentiated compartments. Ultimately, this research seeks to determine the genes and pathways involved in the transdifferentiation mechanism and potential specific biomarkers of these tumors. In order to unravel the mechanisms that drive metaplastic tumors, we studied spatial transcriptomics data (10x Genomics Visium), collected from 8 MpBC patients. Spatially resolved transcriptomics approach captures both histological and transcriptional information. Data were deconvoluted using the RCTD tool from the spacexr R library (4) and annotated by a pathologist. Copy number alterations were computed with infercnvPlus R library(5). MAST R library(6) was used to identify differentially expressed genes. Gene set enrichment analysis was performed to determine changes across pathways(7) .

In the majority of our samples, there is no striking difference between mesenchymal tumor compartments and the epithelial tumoral ones in terms of copy number alterations, suggesting the difference in phenotypes doesn't have a genetic origin. 3 patients out of 8 present significant, contiguous differences between their epithelial and mesenchymal tumor compartments, although their reliability and relevance still need to be further validated. Differential gene expression analysis revealed potential biomarkers highly specific to the spindle cell compartment of MpBC tumors such as the MMP14 or PCOLCE genes. GSEA analysis was performed in order to understand in which biological pathways these genes are involved. It appeared that the significantly upregulated pathways in the mesenchymal compartments are related to the organization of the extracellular matrix.

To conclude, spatially resolved transcriptomics enabled us to come up with new insights on the molecular mechanisms underlying MpBC development, and will enable us to potentially provide

*Speaker

new opportunities for their clinical care.

Keywords: Metaplastic breast cancer, Spatial transcriptomics, Biomarkers

Research to better understand diseases and propose new therapies

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Poster #16

Characterizing the plastic nature of microvilli in *C. elegans*

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Structural plasticity is a key attribute of various cell type in multicellular organism, allowing building of various shapes and structures according to one function. One such highly organized structure is present on the apical side of enterocytes in shape of fingers like protrusion known as microvilli. Despite high organization, microvilli are dynamic in nature and can change their size in response to genetic and environmental changes. Microvilli are crucial for nutrient uptake and perturbation in their structure can result in chronic diarrhea like complications, which can prove to be fatal. We aim to understand the molecular mechanisms dictating the plastic nature of the microvilli and leverage them to develop therapeutic options against various complications involving microvilli. To accomplish these goals, we will use *C. elegans* as our model organism, which presents a very simple intestine made up of only 20 enterocytes. Our first step is to characterize microvillus atrophy in various atrophy models (Toxins, genetic, lectins) we already established, and subsequently the regeneration of these structures. We will combine super-resolution microscopy to study localization of various markers during atrophy and regeneration and electron microscopy for ultra-structural analysis. We hope that our findings contribute to the better understanding of the plastic nature of microvilli and serve as a foundation upon which various therapeutic option for microvillar disease would be developed.

Keywords: Microvilli, *C. elegans*, regeneration

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Research to better understand diseases and propose new therapies

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Poster #17

Phenotypic and Spatial Analysis of Myeloid Cells in a Mouse Model of DLBCL

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Diffuse Large B Cell Lymphoma (DLBCL) is a common, highly aggressive Non-Hodgkin's lymphoma, with a poor prognosis: 30 to 40% of DLBCL patients relapse or are refractory to standard treatments. These failures may be attributed in part to the composition and nature of the tumor microenvironment (TME). In DLBCL patients, the presence and spatial localization of immune cells from myeloid lineage in the TME appear to be prognostic factors (Ferrant J et al., BioRxiv preprint, 2022; Wright et al., Blood Adv, 2023).
Our aim is to determine the phenotypic and functional heterogeneity of myeloid cells infiltrating aggressive B lymphoma in a syngeneic mouse model. In parallel, we are working to map the spatial localization of myeloid populations within the lymphoma and analyze their interactions with T cells and stromal cells of the TME. Our preliminary analyses show that macrophagic populations, dendritic cells, and non-classical monocytes are deregulated in the presence of lymphoma. These results are consistent with analyses of PBMCs and biopsies from DLBCL patients.
Our project will enable us to define the cellular and molecular mechanisms regulating myeloid populations in the DLBCL TME and to identify candidate therapeutic targets that could enhance current clinical treatments.

Keywords: Lymphoma, DLBCL, Myeloid cells, Tumor microenvironment

*Speaker

Research to better understand diseases and propose new therapies

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Poster #18

Detection and Characterisation of Fibronectin Structures in the Tumour Extracellular Matrix

Faisal Jayousi * [1]

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Characterising the tumour extracellular matrix (ECM) holds promise for identifying predictive biomarkers, particularly in assessing patient response to immunotherapy. While the cellular components of the tumour microenvironment have been extensively characterised, the non-cellular elements of this ecosystem remain underexplored. In this study, we investigated the geometry of Fibronectin (FN) in immunofluorescence images, a key ECM protein, in head and neck tumours. Our analysis identified two primary classes associated with FN structure: (a) aligned fibres, and (b) reticular fibre-like, which exist on a spectrum of structural variation. We proposed an approach leveraging Voronoi diagrams as adaptive windows. Circular statistics were then used to capture the spatial organisation of FN, providing insights into its structural heterogeneity within the tumour microenvironment.

Keywords: Fluorescence microscopy, Bioimage analysis, Circular statistics, Spatial tessellations, Extracellular matrix

*Speaker

Research to better understand diseases and propose new therapies

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Poster #19

Regulation of Death Receptor Signalling by UFMylation in Triple-Negative Breast Cancer Cells

Victoria Maltret * [1]

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Signalling induced by the death receptors CD95 and TRAIL-R1/R2 has been described to regulate tumour progression. Ligands of these receptors (CD95L and TRAIL, respectively) can induce cytotoxic pathways leading to the death of target cells. However, death receptors (DRs) can also induce proliferation, cell migration, and cytokine production by activating signalling pathways such as NF-κB and MAPK. Our study investigates the role of UFMylation in regulating death receptor signalling pathways in triple-negative breast cancer cells. The ubiquitin-like modification UFMylation, catalyzed by the enzyme UFL1, is emerging as a crucial post-translational modification involved in the modulation of cellular processes. We aim to explore how UFMylation modulates CD95 and TRAIL-R1/R2 signalling, affecting downstream processes such as apoptosis, cell survival, and inflammatory responses in breast cancer cells.

Keywords: DR, CD95, TRAILR1, TRAILR2, DISC, UFMylation, UFL1, MAPK, NF-κB, Caspase 8, Apoptosis

*Speaker

Research to better understand diseases and propose new therapies

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Poster #21

Study of the Tumor Immune Microenvironment in Human Hepatocellular Carcinoma Using Innovative Multiplex Imaging Technology

Margaux Delaporte * [1], Leïla Leblond-Bouillette [1], Anthony Sebillot [2], Bruno Turlin [3,4], Pascale Bellaud [2], Michel Samson [1], Céline Raguénès-Nicol† [1]

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Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disease, and its prevalence is increasing rapidly in Western countries as a result of alcohol abuse and high-calorie diet. The severe form of MASLD can progress hepatocel- lular carcinoma (HCC), the fourth leading cause of cancer-related death worldwide. Several experimental models have demonstrated the key role of immune cells in the pathogenesis of HCC. However, there is considerable inter- and intra- tumor heterogeneity in this immune environment. Because of this heterogeneity, innovative targeted therapies such as immune checkpoint inhibitors are facing highly variable response rates. To understand this variability and offer the right therapeutic proposal for each patient, it is important to characterize the tumor microenvironment according to aetiology. We wish to determine whether patients with metabolic steatosis having negligible, moderate or excessive alcohol consumption will display a different state of the immune system, which could justify different therapeutic management. Multiplex immunofluorescence (mIF) is an emerging technology developed to overcome con- ventional histology's constraint, enabling the simultaneous detection of numerous markers on a single section of tissue. This project aims to develop an immunofluorescence multiplex panel on FFPE tissue using an innovative technology, CellDIVETM enabling spatial map- ping of the immune infiltrate in human liver. This technology is based on iterative labelling cycles separated by chemical inactivation of the fluorescence. Here we present the panel of markers and immunofluorescence images of HCC obtained using CellDIVETM, enabling us to characterize tissue structure, myeloid and lymphoid cells and tumor development. We will apply it to a biobank of livers from patients with HCC with metabolic dysfunction back- ground and stratified according to alcohol consumption. This cohort will be used to define a signature of the immune infiltrate based on the number of cells of different subtypes and their spatial arrangement.

Keywords: Immunofluorescence, Microenvironment, Hepatocellular carcinoma, Multiplex

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Poster #20

Development of Biosensors for Screening Trans-translation Inhibitors in *Pseudomonas aeruginosa.*

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The World Health Organization has identified *Pseudomonas aeruginosa* as one of the bacteria for which there is a critical need to develop new antibiotics (1). In this bacterium, the deletion of the ribosome rescue pathway, known as trans-translation, has been shown to increase antibiotic susceptibility and decrease tolerance (2). Consequently, trans-translation emerges as an attractive target for novel antimicrobial development (3,4). We present the development of a system designed to facilitate simple and reproducible *in vivo* screening of antimicrobial compounds specifically targeting trans-translation in *P. aeruginosa*. To achieve this, the *hemH* gene has been engineered for the conditional degradation of HemH-ferrochelatase within the cell. HemH catalyzes the conversion of red fluorescent protoporphyrin IX to heme. The system operates such that, when the trans-translation system is active, protoporphyrin IX accumulates, generating a detectable signal. Conversely, inhibition of trans-translation leads to HemH persistence within the cell, resulting in a reduction of the signal. This system offers multiple benefits: measurements are conducted on whole cells, the system does not confer antibiotic resistance, and it is extendable to other emerging pathogenic bacteria. Armed with this *hemH*-based detection system, we aim to discover molecules capable of inhibiting trans-translation to enhance the susceptibility of *P. aeruginosa* to clinically used antibiotics.
Grants: "Vaincre la mucoviscidose" ; "Association Gregory Lemarchal"

References:

1 Reyes J et al. Lancet Microbe. 2023 Mar;4(3):e159-e170.

2 Ren H, et al., J Infect Dis. 2019 Oct 8;220(10):1667-1678.

3 Campos-Silva R et al., Microorganisms. 2021 Dec 21;10(1):3.

4 Guyomar C, et al., Nat Commun. 2021 Aug 13;12(1):4909.

Keywords: Pseudomonas aeruginosa, trans, translation, antimicrobial resistance

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Poster #22

Development of canine tumoral cell lines as therapeutic models in comparative oncology

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The canine species comprises over 400 breeds, the result of artificial selection imposed by humans over the last 10,000 years. As a result, each breed corresponds to a genetic isolate, and in the majority of breeds we observe the segregation of numerous genetic diseases with racial predispositions, such as cancers and diseases that are rare in humans. The Dog Genetics team works on canine cancers as genetic and therapeutic models for homologous human cancers.
We have developed tumoral cell lines from canine tumoral tissue (mucosal melanomas, histiocytic sarcomas, osteosarcomas, gliomas, etc.), characterized them genetically, and tested their tumorigenicity in immunodeficient mice, in collaboration with Biotrial, as part of the LabCom Oncotrial program. Finally, we carried out response tests to a dozen drugs, using proliferation assays and *in vitro* IC50 definitions.

We have also genetically modified certain potential oncogenes discovered in dogs, using the CRISPR-Cas9 technique, with the aim of demonstrating their role as driver oncogenes in the lines of interest. The modified cell lines are implanted in mice, and their proliferation and dissemination characteristics are compared with those of the cell line not carrying the mutations studied.
These results will enable us to identify well-characterized tumoral cell lines with characteristics homologous to human cancers, which can be used to test new drugs. The cell lines can also be genetically modified according to the genes targeted by the drugs to be tested, offering a wider choice of tools for testing new therapies.
To date, a panel of some twenty canine tumoral cell lines has been established, providing a relevant tool for oncology studies with a double benefit for humans and dogs.

Keywords: Cancer, Genetics, Dog, Mucosal melanoma, Histiocytic sarcoma

*Speaker

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Poster #23

Synergistic Effects of IRE1 Inhibition on Standard of Care in Glioblastoma Mouse Models: Potential Mechanisms of Action

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Glioblastoma (GB) is a highly aggressive brain tumor with a poor prognosis, leading to a median survival in patients of around 15 months. Current treatment, called the "Stupp protocol," is insufficient, highlighting the need for novel therapeutic approaches. We identified IRE1, a sensor of the Unfolded Protein Response, as a promising therapeutic target in GB. IRE1 activity correlates with GB aggressiveness, and genetic ablation of IRE1 in tumor cells significantly increased survival in preclinical models. To investigate the clinical potential of IRE1 pharmacologic inhibition in GB, we developed a syngeneic GB mouse model based on orthotopic injection of GL261 murine glioma cells treated with an adapted Stupp protocol. Using this model, we demonstrated that inhibition of IRE1 with an RNase inhibitor (MKC8866) sensitized GB to the Stupp-like regimen. These results were confirmed in another mouse model (xenogenic, U87) and with another IRE1 inhibitor targeting kinase activity. To identify the mechanisms by which IRE1 inhibition could enhance the efficacy of the standard of care in GB, we performed whole exome sequencing analyses on isolated tumor cells (GL261) or bulk tumors (U87). Consistent results in both models indicated that only tumors treated by a combination of standard of care and IRE1 inhibitors exhibited signs of defective DNA repair mechanisms. Thus, IRE1 inhibition might reduce DNA repair ability in GB cells, sensitizing them to genotoxicity. Transcriptome analyses of the same samples revealed that DNA repair mechanisms are among the most upregulated pathways upon IRE1 inhibition. In conclusion, our work provides evidence of functional links between IRE1 signaling and DNA repair in vivo and opens new avenues for modulating IRE1 signaling as a therapeutic approach in GB and other diseases.

Keywords: IRE1, Glioblastoma, Whole Exome Sequencing, MKC8866, Multiomics

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Poster and flash-talk #24

Atrophy and regeneration of the intestinal brush border

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The intestine epithelium is organized in a crypt-villus axe. This wave-like organization increases the exchange surface of the tissue with the intestine lumen. Additionally, it creates niches at the base of the villus where stem cells can proliferate and differentiate to renew the epithelium, the crypts. The major type of cell generated by the crypts is the enterocytes, in charge nutrient absorption. To do so, those polarized cells display at their apical side a brush border. This brush border is made of cell protrusion named the microvilli, present all along the intestinal epithelium. Unfortunately, this brush border can be altered by a rare genetical disease:The Microvillus Inclusion Disease (MVID).This medical condition causes the atrophy of the microvilli, leading to severe chronic diarrheas and a decrease of the nutrient absorption.Those malfunctions are fatal for the patient at an early stage of life. Beside this chronical atrophy, brush border can also experience acute atrophy caused by different stresses: alimentation, chronic inflammation, etc.

If the cause and consequences of MVID are well known, the molecular mechanism leading to the atrophy of microvilli remains unclear. Furthermore, the highly proliferative nature of the intestine epithelium coupled with the short lifespan of enterocytes raised and important question:Do cells regenerate the brush border or are they replaced in an acute atrophy context?

Using intestinal mouse organoids and classic cell culture, the goals of my project is to help understanding how does the atrophy of microvilli occur at the molecular level. But also, to study the potential regeneration of the brush border.For the present, we have identified leads that could help understanding the mechanism behind atrophy. Moreover, we have obtained preliminary results suggesting that regeneration of the brush border is possible *in vitro*.

Keywords: Microvilli, Regeneration, Atrophy, MVID, Cellular biology

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Poster #25

Implication of the RNA-binding protein CELF1 in the regulatory mechanisms of cytoskeleton dynamics during lens development

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In mice, the inactivation of the **Celf1** gene leads to cataracts as well as disorganization of the lens structure, linked to cytoskeletal alterations. The objective of my work was to characterize the changes affecting cytoskeletal dynamics and the signaling pathways induced by the loss of **Celf1** expression.
My results, obtained from 21EM15 mouse lens cells, show that CELF1 depletion alters their morphology, cytoskeleton, and migratory abilities. Cells expressing an sh-RNA targeting Celf1 (sh-Celf1 cells) exhibit a loss of elongation, formation of large lamellipodia associated with cell polarization, and reduced migratory capacity, likely related to an increased number of focal adhesions. While control 21EM15 cells display characteristics suggesting a mesenchymal state, sh-Celf1 cells seem to shift towards a more epithelial phenotype. Furthermore, iCLIP data from the team, along with my literature research and western blot experiments, indicate that the non-canonical Wnt pathway may be involved in these phenomena.
Our results suggest that CELF1 may play a role in the differentiation of lens epithelial cells into lens fibers through an EMT process, by modulating Wnt pathway activity. The involvement of such signaling pathways in the development of cataracts could therefore lead us to new drug therapies against cataract.

Keywords: Cataract, signal pathway, Wnt, EMT, cytoskeleton

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Poster and flash-talk #27

Identification of new anticancer drug candidates from the Prestwick chemical library: comparative effects on gastric cancer cells in 2D vs. 3D

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Gastric cancer (GC) is the fourth leading cause of cancer-related death. The incidence of GC in Finistère is the highest in France. Often detected at an advanced stage, the prognosis for GC patients remains poor, even though many drugs exist, but they are poorly efficient.
Given this situation, our team explored drug repurposing. A pharmacological screening has been initiated (George Azeeb) using the **Prestwick chemical library**, made up of 1520 drugs already available on the market and covering a wide range of therapeutic indications. The screening was conducted on **spheroids** (3D) cultures developed from the HGT-1 gastric cancer cell line. This initial screening identified 90 molecules (MTT assay) that reduced cell survival to less than 25%. Subsequently, 19 of these 90 molecules were tested in both 2D and 3D cultures using HGT-1 and AGS (another human gastric cancer cell line) and HEK293 (embryonic kidney cell line) spheroids used as a non-cancerous control. Three molecules, not used in cancer treatment, demonstrated distinctive cytotoxicity when used as single agents. This ranged from 51 to 94 % depending on the cell line, after 48h of treatment in 2D models. The effects of their combination with several reference chemotherapies were evaluated in both 2D and 3D models, revealing a **significant synergistic activity** between one identified molecule with 5-fluorouracil (5FU), increasing toxicity from 40% with 5FU alone to 80% when combined. RNAseq analyses to compare the overall gene expression profiles between 2D and 3D-grown gastric cancer cells, and in response to treatments are being done. Further analyses are ongoing to determine the specific type of **cell death** triggered by these agents, including apoptosis (likely), necroptosis or ferroptosis.
Finally, will next analyse the effects of these drug combinations in **bi-cellular spheroids** that associate epithelial cancer cells with **cancer-associated-fibroblasts** (CAFs), with more relevance to tumors.

Keywords: Gastric cancer, Drug repurposing, Drug screening, Spheroids

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Poster #28

Exploring mitochondrial cristæ as functional and dynamic hubs of the kinase AURKA

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Mitochondria are key organelles in cellular homeostasis, and are often deregulated in cancer. Among the key features of breast cancer conditions, the multifunctional Ser/Thr kinase Aurora A/AURKA is frequently overexpressed. The auto-phosphorylation of AURKA on Thr288 allows the kinase to enter into mitochondria and to localize at the matrix. Overexpression of AURKA causes a deregulation of mitochondrial ATP production, morphology and the degradation of defective organelles by mitophagy. Our previous studies obtained with Förster's Resonance Energy Transfer (FRET) unraveled a strong interaction between AURKA and Prohibitin 2 (PHB2), one of the key *cristae* proteins. We showed that this interaction allows AURKA to orchestrate mitophagy.
Our research focuses on the characterization of the interactors of AURKA and PHB2 within mitochondria. Our proteomics-based studies identified that these baits are interacting with the same mitochondrial *cristae* proteins. We are studying what impact AURKA overexpression can have on this pool of proteins, and whether by acting on this platform we may counteract the mitochondrial dysfunctions induced by AURKA overexpression.

In AURKA-overexpressing cells, AURKA uses mitophagy to select a pool of mitochondria with high ATP production rates. Mitophagy can be partially rescued using capsaicin, a specific PHB2 inhibitor. Our recent results indicate that the AURKA-PHB2 interaction is strongly reduced in the presence of capsaicin, which also restores mitochondrial mass and morphology.
Our results suggest that AURKA uses *cristae* proteins as a platform to hijack mitochondrial functionality and to sustain cell proliferation. They also pave the way to the targeting of the AURKA-PHB2 interaction as a pharmacological strategy to revert the consequences of AURKA overexpression in cancer conditions.

Keywords: Mitochondria, Cancer, AURKA, FRET/FLIM Microscopy, Interactomics

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Poster and flash-talk #29

Insight into new forces involved in mitotic spindle positioning

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Accurate mitotic spindle positioning, an MT-based machine, is essential for proper cell division, tissue organization, and cellular function. During asymmetric SC (Stem cell) division, spindle orientation ensures uneven distribution of cortical fate determinants in the daughter cells. As a consequence, one cell is subjected to differentiation while the other is keeping the SC identity. In this Drosophila neural SC, defects in spindle orientation along the apico-basal axis of SCs can disrupt the asymmetric distribution of cell fate determinants. As a consequence, both daughter cells may acquire the SC identity, which is associated with the formation of aggressive tumors. The canonical model proposes that spindle positioning is governed by pulling forces generated by the Dynein motor, located at the apical cell cortex on the astral microtubules (MT) that are nucleated by the centrosomes. However, by analyzing spindle position in fly NSCs with normal or altered spindle shapes, we observed a predominance of spindle position towards the basal cortex. Our findings suggest that novel MT-based pushing forces against the apical cortex and/or MT forces pulling emanating from the basal cortex contribute to spindle positioning. In order to characterize these new forces, we have adopted several strategies to dissect these forces, including genetic and optogenetics manipulation of centrosomal MT. Our preliminary results demonstrate the tool's functionality and its potential to dissect force dynamics *in vivo*. This research offers new insights into the molecular mechanisms of spindle positioning and opens up new avenues for understanding asymmetric cell division processes.

Keywords: Spindle positioning, Centrosome, Astral microtubule, forces, Neural stem cells optogenetic, Drosophila

*Speaker

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Poster and flash-talk #30

Identification of the role of circRNAs in BRAFi resistance in metastatic melanoma treatment

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50% of the patients diagnosed with metastatic melanoma harbor the driver mutation V600E of the kinase BRAF, resulting in an uncontrolled cell growth. BRAF inhibitors (BRAFi) combined with MEK inhibitors (MEKi) are the main treatment in this case. However the majority of patients develop resistance. This resistance is linked to melanoma plasticity, which can be influenced by various noncoding RNAs, including long noncoding RNAs and circular RNAs (circRNAs). CircRNAs are abundant in the eukaryotic transcriptome and can act as miRNA sponges, preventing miRNAs from degrading their target mRNAs.

This project aims to decipher the role of the circRNA-miR-mRNA network in regulating BRAFi resistance genes in melanoma using comprehensive bioinformatics approaches. We particularly focused on the regulation of critical metastasis and BRAFi resistance regulators such as AhR, AXL, and EGFR. Using luciferase reporter assays, we identified miR-MRE interactions on the 3'UTRs of AhR, AXL, and EGFR, as well as on circRNAs.

Parallel overexpression of specific miRNAs through transfection of synthetic miRNA mimics led to the specific downregulation of AhR and AXL, as confirmed by qPCR and Western blot analysis. Additionally, depletion of specific circRNAs, such as circ_443 and circ_2805, resulted in decreased levels of AhR and EGFR.

We further explored the impact of each circRNA and miRNA on BRAFi sensitivity (sensitivity assay) and metastatic phenotype switch (spheroid assay) to propose new therapeutic strategies.

Keywords: Melanoma, AhR, BRAFi, circRNA, miRNA

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Poster and flash-talk #31

Integration of the IL4 signaling during polarization of CAF in follicular lymphoma: implication of the STAT pathway.

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Follicular lymphoma (FL) is an incurable and indolent liquid cancer originating from B cells, localized in the lymph nodes and invading the bone marrow in 70% of cases. In France alone, between 3000 and 5000 patients are diagnosed each year and 10% of patients do not survive up to 5 years after diagnosis, with relapses more and more frequent after 5 years in survivors. The scientific literature on FL is currently sparse and it is still unknown why some patients develop a more aggressive form of FL and why current treatments are unable to cure this cancer. The tumor microenvironment (TME) is increasingly recognized as a critical factor in cancer prognosis and outcome. Since the TME in FL is not fully understood yet, we investigated the transition from resident lymphoid stromal cells (LSC) to cancer-associated fibroblasts (CAF). To study the interaction between stromal and cancer cells, we developed an*in vitro*model of normal and pathological LSC commitment. Briefly, progenitor cells were polarized into LSC-like cells by TNF-alpha and lymphotoxin. These cells were then further committed to CAF, by the addition of additional cytokines such as IL-4. In this model, by analyzing the transcriptomic signatures obtained in vitro, as well as transcriptomic data of native stromal cells (both healthy and FL patients) we discover that IL-4 signaling is integrated through the activation of STAT1 and STAT2 pathways*in vitro*and that these pathways are upregulated in stromal cells from FL patients. Moreover, interactome analysis suggests that IL-4 signaling through STAT1 pathway activation could favor the production of FL-B cells survival signals. Collectively, our work reveals a previously unknown implication of IL-4 in CAF polarization and highlights this pathway as a potential new therapeutic pathway to suppress the emergence of pro-tumorigenic CAF.

Keywords: follicular lymphoma, cancer associated fibroblast, gene regulatory network, IL4, STAT

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Poster and flash-talk #32

LOS inhibition in *A. baumannii* causes sensitivity to josamycin: a new combination therapy approach using LOS inhibitors and antibiotics in multiresistant *Acientobacter baumannii*

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Josamycin, like many other antibiotics, is impermeable to the outer membrane of gram-negative bacteria including *Acinetobacter baumannii* an opportunistic pathogen whose importance has increased due to the prevalence of multiresistant strains in hospitals. In the fight against antimicrobial resistance, the fact of not being able to use antibiotics that can penetrate the cell and reach their intracellular target is a disadvantage, since the bacteria are increasingly becoming more resistant, thus reducing the therapeutic options available. Here, we found that josamycin, an unusual clinical antibiotic against *A. baumannii* that may inhibit the growth of bacteria when it is used in a combination therapy with a lipooligosaccharide (LOS) synthesis inhibitor. We identify antimicrobials that act synergistically with inhibitor of LpxC, one of the enzymes involved in LOS biosynthesis in multiresistant *A. baumannii*. For this purpose, a sophisticated study model was validated by confronting the library compounds against the strain ATCC 19606 and a strain without LOS. Then the type of interaction between these compounds and the LpxC inhibitor CHIR-090 in the wild-type strain and MDR clinical isolates were tested by means of checkerboard assays and lethality curves. Therefore, *in vitro* inhibition of LpxC increases *in vitro* permeability to cefotaxime, cefixime and josamycin. Finally, the set of data presented indicates that combination therapy is one of the alternatives to combat antibiotic resistance in bacteria.

Keywords: Antibiotic resistance, Double theraphy, LPS

*Speaker

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Poster and flash-talk #33

Prevalence and Characterization of Infectious Relapses due to *Enterobacter cloacae* Complex

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Objective and Introduction: Infectious recurrences can be attributed to two phenomena: 1) reinfection by a new bacterial strain or 2) relapse, where different episodes are caused by the same persistent strain. Recently, the concept of bacterial persistence has emerged, and its involvement in relapses is strongly suspected. The objectives of this project were to estimate the prevalence of relapse in infections caused by-*Enterobacter cloacae*-complex (CEC) through a comparative genomic analysis (CGA) from sets of clinical isolates responsible for infectious recurrences in each patient. Additionally, the study aimed to identify metabolic pathways potentially involved in bacterial persistence through single nucleotide polymorphism (SNP) analysis.
Materials (or Patients) and Methods: Clinical isolates were retrospectively collected between January 2017 and January 2024 at the University Hospital of Rennes. Patients with at least two episodes of invasive infections - such as bloodstream, intra-abdominal, bone and joint or respiratory - caused by CEC were included in the study. The genomes of these clinical isolates were sequenced (BGI; 2 x 150 bp protocol), and a CGA was performed using the CLC Genomics Workbench software (Qiagen).
Results: Overall, 21 patients were included, with a total of 50 isolates and 29 recurrence episodes. Most samples originated from bone and joint infections (19/50, 38 % and from bacteremia (15/50, 30 %. CGA revealed that relapse occurred in 19 patients (90.4 %, thus representing 86.2 % of recurrence episodes. Furthermore, SNPs were identified in genes related to the RCS system, which regulates bacterial capsule synthesis, for 3 patients. These mutations were associated with modification in the mucoid phenotype of several isolates. Conclusion: Our findings underscore the significant role of relapses in infectious recurrences, accounting for over 85 % of recurrent CEC infection episodes. The common genetic signals observed across different patients suggest that the bacterial capsule may play a crucial role in these infectious relapses.

Keywords: *E. cloacae* complex, Persistence, Relapse, RCS system

*Speaker

In silico Biology

Synthetic DNA as the future of data archiving - Technological advances and challenges

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MédiaCoding Team

Marc Antonini is a CNRS research director at the Computer Science, Signals and Systems laboratory in Sophia-Antipolis (I3S), where he heads the MediaCoding team.

He specializes in data compression, whether for images, videos or 3D models. His doctoral work, for example, was used for the JPEG 2000 standard, and his early work at CNRS, in collaboration with CNES, for one of the systems onboard the Pleiades satellites (a pair of optical Earth observation satellites). The author of thirteen patents, Marc Antonini has regularly collaborated with various manufacturers and co-founded the start-up Cintoo, dedicated to the capture and visualization of 3D point clouds. Since then, he has turned his attention to synthetic DNA storage. Marc Antonini heads the MoleculArXiv research program (PEPR), endowed with twenty million euros over seven years to develop this technology of the future, and has participated in the European OligoArchive program. On the same theme, Marc Antonini co-founded the start-up PearCode and presided over the design of JPEG DNA, an image compression standard adapted to DNA.

Marc Antonini received the CNRS Innovation Medal in 2023.

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In silico biology

Short-talks

ANNEXA: A Comprehensive Pipeline for Extending Genome Annotations Using Long-Read Transcriptome Sequencing

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In recent years, the development of long-read transcriptome sequencing has allowed the sequencing of whole transcripts, giving a better view of repeated regions and direct exon/exon connectivity which are often inaccessible with traditional short-read sequencing. This allows us to have an unfragmented vision of the transcriptome and thus should improve genome annotation. However, the development of these long-read sequencing technologies also requires the development of new bioinformatics tools.

To this end, we have developed ANNEXA, an all-in-one reproducible pipeline written in the Nextflow workflow management language that extends user-provided reference annotations from long-read sequencing data. ANNEXA works by using only three input files: a reference genome, a reference annotation, and a file listing the transcripts to analyse. It reconstructs transcripts and quantifies their abundance, with the choice of two different transcript reconstruction tools: Bambu and Stringtie2. ANNEXA then predicts whether novel identified transcripts are long non-coding RNAs or protein-coding before predicting the coding sequence of the latter RNAs. Finally, novel transcripts are filtered based on their likelihood to correspond to full-length transcripts using deep learning models trained to validate novel transcription start sites, before being added to the final extended annotation. A final quality control process produces a graphical report, allowing users to quickly obtain information such as the number of novel genes, isoforms and exons at the lncRNA and mRNA levels.

To demonstrate the robustness of ANNEXA, we have tested its ability to extend reference annotations using data from both Oxford Nanopore Technologies and Pacific Biosciences sequencing. In the context of comparative oncology studies, we have applied ANNEXA to ONT data from 2 human and 7 dog cancer cell lines, showing its ability to extend annotations from different organisms.

Overall, our work presents a new bioinformatic pipeline to automatically reconstruct and characterize mRNAs and lncRNAs from long-read transcriptome data.

Keywords: Genome annotation, Long, read sequencing, lncRNA, Nextflow

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Role of circular RNAs in treatment resistance of cutaneous melanoma.

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Cutaneous melanoma is an increasingly prevalent skin cancer, which is frequently associated with the BRAFV600E mutation. This mutation has led to the development of targeted therapies known as BRAF inhibitors (BRAFi). Unfortunately, resistance to BRAFi often occurs after approximately 18 months of response. To decipher the underlying resistance mechanisms, our study focuses on the role of circular RNAs (circRNAs), a class of non-coding RNAs recently studied for their microRNA (miRNA) sponge activity, which indirectly regulates gene expression. Currently, few bioinformatics tools are available to explore such mechanisms. Therefore, we developed Cirscan, an interactive application that identifies circRNAs acting as miRNA sponges under specific biological conditions on a large scale using transcriptomic data (Fraboulet *et al.* 2024). Cirscan identifies and ranks each circRNA-miRNA-mRNA network according to a sponge score that integrates multiple reliability criteria, where the top-ranked networks represent the most relevant circRNA sponge candidates. The performance of Cirscan has been validated on two public datasets, in which it successfully identifies validated and published mechanisms enriched among the top candidates.

Using transcriptomic data generated by the GEO team at IGDR, including both coding and non-coding RNAs from cutaneous melanoma cell lines resistant or sensitive to treatment, Cirscan identified over 150 circRNA-miRNA-mRNA interaction networks. Among these, about ten sponge mechanisms involving key players in BRAFi resistance in cutaneous melanoma have been experimentally validated, opening opportunities for the development of new therapeutic strategies against treatment resistance.

Keywords: Circular RNAs, Sponge mechanism, Transcriptomic data, Regulation network, Treatment resistance, Cutaneous melanoma

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Structural Study of the Hibernating Ribosomes in Pathogenic Bacteria

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Ribosomes are the universal molecular machines responsible for protein production by translating the genetic code carried by messenger RNAs into proteins. They may exist in different functional states in the cell. One of such states is ribosomes in inactive hibernating state, ubiquitously present within the bacterial domain as well as in all three domains of life. In bacteria, the ability to enter a dormant state of life through ribosome hibernation plays a crucial role in their survival in various stress environments, including ribosome-targeting antibiotics attack. This phenomenon takes place by storing ribosomes in an inactive form through binding of hibernation factors in their active sites, including ribosomal drug-binding sites. This will not only shut down the translation process but also allow the bacteria to resume the protein production promptly as soon as favorable conditions arose by not going through ribosome dissociation and association pathways. In the current work, we focused on understanding how these ribosomal binding proteins interact with ribosome active sites and how its interaction takes charge in inhibiting the translation in four of the ESKAPEE pathogens. The World Health Organization designated seven "ESKAPEE" pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.,* and *Escherichia coli*) as critical targets for drug discovery. Here we report single particle cryo-electron microscopy structures of *K. pneumoniae, A. baumannii, P. aeruginosa* and *E. cloacae* hibernating ribosomes. Our results provide valuable insights into ribosomal hibernation of these four pathogens, paving the way for better understanding of ribosome dormancy, and explore the possibility of using antibiotics to target not only active but also hibernating ribosomes.

Keywords: Bacteria, Ribosomes, Translation, Bacterial hibernation

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Super-resolution radial fluctuations to investigate the mitochondrial morphology in AURKA over-expression

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Mitochondria are defined as the powerhouses of the cells, but they are also involved in fatty acid and calcium regulation, and reactive oxygen species signaling. These organelles form a dynamic network with morphological modifications through fusion, fission, and mitophagy. Recently, studies have revealed that the protein kinase Aurora A (AURKA), known to be overexpressed in epithelial cancers, is imported into the mitochondria and induces mitophagy1. While the kinase was shown to interact with mitochondrial fusion and fission proteins, its role on mitochondrial morphology under mitophagy-inducing conditions remains to be established. To this end, we used a super-resolution (SR) strategy to investigate the morphological changes of mitochondria during AURKA-induced mitophagy, Super-Resolution Radial Fluctuations (SRRF)2. To validate our methodology, we overexpressed both an active and a kinase-dead form of AURKA uncapable of performing mitophagy, and we labeled the mitochondria using MitoTracker Deep Red. We acquired low-resolution images with a spinning-disk microscope, and we used these images to quantify mitochondrial length and branching on raw and super-resolved SRRF images. First, SRRF allowed us to obtain SR images of the mitochondrial network. While the morphological analysis images at low resolution did not show any decrease in branching or length, the analysis of SRRF images showed that the overexpression of active AURKA reduces the length and branching levels. Overall, we shown that AURKA induces mitochondrial fission in mitophagy-prone conditions. Last, our work paves the way for the application of SRRF to the detailed study of organelle morphology.

1. Giulia Bertolin *et al.* Mitochondrial Aurora kinase A induces mitophagy by interacting with MAP1LC3 and Prohibitin 2. *Life Sci. Alliance*, (2021).
2. Gustafsson, N. *et al.* Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations. *Nat. Commun.*, (2016).

Keywords: Super resolution microscopy, Mitochondria, AURKA

*Speaker

In silico biology
Posters and Flash-talks

***In silico* biology - Poster #7**

A pipeline using knowledge graphs to predict gene-phenotype association in different species

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In systems biology, combining different datasets from various sources is relevant for understanding complex biological interactions. We have created a versatile pipeline using Nextflow to predict gene-phenotype associations. It relies on knowledge graphs generated from *C. elegans* (Wormbase) and human data (HGNC, HPO, GO, STRING, Reactome etc.).
Our pipeline uses Semantic Web technologies1, especially SPARQL endpoints, to manage large multimodal biological datasets such as gene-phenotype association, gene-disease association, protein-protein interactions, gene expression data, gene-ontology association and their related ontologies. It was implemented with FAIRness in mind allowing data traceability and version control, and results reproducibility. Our method involves selecting and formatting data through SPARQL queries and using machine learning embedding techniques to turn these graphs into vector representations. Although we developed models with good convergence during training, our early gene-phenotype predictions were not conclusive. These results suggest issues with data structure and/or quality or inappropriate embedding strategies.
In summary, we developed a scalable and adaptable pipeline for predicting gene-phenotype associations. In the future, we will focus on refining data representation and formats to improve predictions, exploring more embedding models from the literature, and adding more human data types to make our approach more robust.

Keywords: Knowledge graphs, Semantic Web, Link prediction, Reproducibility, Nexflow

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Introducing nf-core/phaseimpute -r dev from idea to release

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Genome imputation is a statistical technique that enhances the resolution of genotyping arrays and low-pass sequencing (< 1x) by filling missing data with information from reference panels. While existing pipelines primarily focus on the imputation step and in the human species, crucial steps such as panel preparation, phasing, and imputation assessment are often overlooked.

To address this gap, we introduce nf-core/phaseimpute, a comprehensive pipeline performing panel preparation, genetic simulation, imputation, and tool assessment. Each step is designed for independent execution, enabling users to save outputs and computational time for subsequent analysis. In addition, we took advantage of Nextflow's capabilities in workflow distribution by processing each dataset by chromosomes or chunks. This means that tasks can be processed in parallel, reducing overall execution time. With support for various imputation tools like GLIMPSE1, GLIMPSE2, STITCH, and QUILT, the pipeline accommodates diverse research needs. Moreover, it offers flexibility by allowing execution with or without reference panels, making it invaluable for non-model species where phased haplotypes may not always be available.

The journey from the initial idea to the first release of the nf-core/phaseimpute pipeline in the nf-core community has been an extensive one. Starting with the aim of creating an efficient, reproducible solution for genomic phasing and imputation, we developed the essential imputation and phasing processes within the nf-core modules repository before integrating them into subworkflows. Using the existing nf-core modules significantly accelerated the development process. Throughout the implementation, we observed that some nf-core modules required design modifications when first tested within the pipeline context. These adjustments were necessary to accommodate all required parameters and ensure the modules met the user's specific requirements. Consequently, we contributed back to the community by adding new functionality that was not previously available. Additionally, we benefited from advancements made in Nextflow plugins, such as nf-validation and nf-test. The nf-validation plugin enabled us to enforce schema validation, ensuring that our pipeline configurations met predefined standards and reducing the likelihood of errors. Continuous integration testing and the nf-test plugin were used to verify that each update maintained the pipeline's accuracy and stability, ensuring that no matter the changes different developers would make in the code, the final output files would still be the same. Collaborative efforts within the bioinformatics community facilitated the integration of optimal tools and rigorous testing, resulting in a reliable, high-performance pipeline now accessible to the nf-core community for advanced genomic research.

Keywords: Imputation, FAIR, Open, Science

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Performance Benchmarking of circRNA Annotation Tools using Nanopore Sequencing Data

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Background
Circular RNAs (circRNAs) have emerged as a promising new class of non-coding RNAs (ncRNAs) given that they represent potential stable biomarkers for various traits and diseases. However, their wide size distribution, from under 100 to nearly 100,000 nucleotides, and the presence of multiple isoforms derived from the same linear RNA pose challenges for circRNA analysis using second-generation RNA sequencing technologies. To overcome these limitations, third-generation sequencing, specifically long-read RNA sequencing techniques, are being increasingly employed to capture the full diversity of circRNAs, enhancing our understanding of their roles in biological processes and disease mechanisms.
Methods
This study presents a novel benchmark comparison of four bioinformatics tools—CIRI-long, IsoCirc, circFL-seq, and circNICK-lrs—designed for circRNA detection using long-read nanopore sequencing data. To date, no direct comparison of these tools has been conducted. We designed benchmark datasets based on simulated data and real data for which ground truth was based on experimentally validated circRNA isoforms and union of circRNA databases. We applied the same dataset to all four tools, aiming to provide practical insights into their effectiveness and usability. Our analysis offers guidelines for selecting the most efficient methodology for circRNA identification based on different research needs.
Key findings
Preliminary results from one benchmark dataset show:

- Among the tools, circFL-seq detected the most isoforms, including the highest number of novel circRNAs.
- circNICK-lrs was the only tool capable of identifying isoforms larger than 5,000 nucleotides, though it requires specific reference genomes and annotation files.
- IsoCirc, while showing the highest performance metrics, identified the fewest isoforms and applies a length cut-off in its default settings.

Keywords: circRNA, long read nanopore sequencing, bioinformatics tools, benchmark comparison

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Association and impact of genomic point mutations and structural variations on canine longevity

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Humans have intentionally bred dogs for specific traits, transforming them into a valuable mammalian model for studying the genetic foundations of various phenotypes, diseases, and longevity. Our team focuses on investigating the genetic and epigenetic factors underlying lifelong traits in dogs, using them as a natural research model. In our exploration of the canine model's potential in studying longevity , we observe an inverse relationship between a breed's average lifespan and its size, with smaller breeds typically living longer. Building on this, we're now working to develop a comprehensive database of genetic variations across 25 dog breeds. This project involves low-coverage DNA sequencing of 500 aged dogs, long-read DNA sequencing for 100 canines, and creating genome assemblies for a diverse panel representing the primary breeds. This extensive genetic resource will not only advance our understanding of genomic diversity in dog populations but also introduce a novel approach for mapping genetic variations to specific phenotypes.

Keywords: Dog model, Longevity, Longread DNA sequencing

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Integrating prior knowledge networks with context-specific transcriptomic data to study the effect of SHH pathway defects in neurodevelopment

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Holoprosencephaly (HPE) is a rare neurodevelopmental disorder caused by a reduced activity of the Sonic Hedgehog (SHH) signaling pathway. This disease is characterized by significant heterogeneity both in terms of phenotype and genetic causes. As SHH is the main gene involved, 18 genes are known to play a role in HPE by reducing SHH morphogen activity. This genetic complexity leads to a low rate of precise molecular diagnosis (30%) that emphasizes the need to better understand the molecular interplay occurring in HPE.
However, the inaccessibility of the primary affected tissue in patients constitutes one of the main challenges in the study of HPE. To overcome this, we developed an *in-vitro*-based model of human developing neuroectoderm using induced pluripotent stem cells (iPSCs). We performed 3'RNA-seq analysis on this model, giving us insight into the molecular consequences of SHH deficiency on the key tissue affected in HPE.
Beyond the statistical methods relying solely on these context-specific data, such as differential expression or co-expression analysis, we integrated them with prior knowledge networks. By doing so, we can further study the effect of the SHH defect on key developmental regulators such as transcription factors and dissect active gene modules identified from multiplex networks.

Keywords: neurodevlopement, SHH, Holoprosencephaly, transcriptomic, network, transcription factors

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Simulation of data-driven multi-omic benchmark data for cellular deconvolution methods evaluation

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Data-driven simulation is extensively used to assess the performance of statistical methods in a large variety of conditions. Indeed, *in vivo* or *in vitro* experiments with ground truth in controlled conditions are too expensive to produce a large number of replicates, especially in genomics. Similarly, designing a genomic data challenge such as HADACA3, whose focus is on multi-omics cellular deconvolution, also requires to reproduce in silico the complex variability of paired bulk tissues from many omics data types. Here we focused on two frequently used omic data types for cell deconvolution, RNA-seq gene expressions data and DNA methylation rates. Most stochastic models for such multivariate data assume specific distributions at the individual level, CpG's probes or genes, consistently accounting for the intrinsic nature of genomic measurements, including nonnegativity and overdispersion. However, introducing biologically sound patterns of dependence, across genes or, for a given gene, across different types of omic data, is more challenging, due to the complexity of this dependence and the huge number of genes or CpG sites. Ignoring this dependence in simulation setups may lead to a strong optimism bias in the evaluation of cell deconvolution methods.
The gene regulatory network induces a particular dependence structure for both data types, which for this study is inferred from*in vitro*benchmark gene expression and DNA-methylation data using an efficient Expectation-Maximisation algorithm (1). Here, we propose a data-driven methodology introducing a low-rank factor approximation of the conditional variance-covariance between genes. The factorial decomposition captures specific and shared variability of the data, with different levels of details possible controlled by the number of factors treated as a tuning parameter.
Our study clearly demonstrates that the accuracy of most cell deconvolution algorithms strongly depends on the interplay between patterns of cellular heterogeneity and dependence across genes.
(1) Friguet, C., Kloareg, M., & Causeur, D. (2009). A Factor Model Approach to Multiple Testing Under Dependence. *Journal of the American Statistical Association*, *104*(488), 1406–1415. https://doi.org/10.1198/jasa.2009.tm08332

Keywords: Simulation, Data challenge, Cell deconvolution, Multi, Omic data integration

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DeCovarT, a Multidimensional Probabilistic Model for the Deconvolution of Heterogeneous Transcriptomic Samples

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Although **bulk transcriptomic** analyses have significantly advanced our understanding of complex diseases, their sensitivity is hindered by the intrinsic heterogeneity of biological samples, since they average measurements over several distinct cell populations. Yet, dissecting tissues at a high granularity level is particularly critical for a comprehensive description of the landscape of **Tumor Microenvironments** (TME), a hallmark of **solid tumor evolution**.

To address this limitation, numerical approaches, known as **deconvolution** algorithms, have been developed to automatically estimate **cellular composition**, typically using reference samples of physically purified populations. However, these algorithms often perform poorly when differentiating closely related or infrequent cell populations.

In contrast to standard linear approaches, which assume no interaction between genes, we hypothesised that explicitly integrating **gene regulatory networks** (GRNs), at the cell population level, could enhance the performance of deconvolution algorithms. We implemented this approach in a new tool, *DeCovarT*, which models pairwise transcriptomic interactions in a generative, multidimensional model, to reconstruct global bulk profiles. Specifically, we inferred cellular abundances using a standard *maximum likelihood estimation* (MLE) approach, employing an *Alternating ECM* (AECM) iterative method coupled with a specific implementation of the *Levenberg-Marquardt* algorithm, to recover the global maximum of the resulting global bulk distribution, conditionned to the individual cellular features. Additionally, we reparameterised the log-likelihood to naturally integrate the *unit-simplex constraint* on cellular ratios. Last but not least, the intrinsic stochasticity of transcriptomic expression is natively incoportated in our model, through describing each cellular biomolecular fingerprint by a multivariate Gaussian distribution. Precisely, a *sparse precision matrix* is inferred using the graphical Lasso algorithm, enabling to straightforward encode the GRN.

Preliminary numerical simulations suggest that this new algorithm outperforms previously published methods in **discriminating closely related cell types**, for which no specific proteomic surface targets or transcriptomic markers are known. The code, along with additional simulation outputs, is publicly available in a **GitHub** repository: https://github.com/bastienchassagnol/DeCovarT. Furthermore, a comprehensive description of the theoretical results, including explicit derivation of analytical formulas for the Gradient and the Hessian of the log-likelihood function, and a description of the reparameterisation technique, is available as a preprint on **arXiv**: https://cnrs.hal.science/hal-04208010. This preprint also discusses a potentially more robust approach: the joint reconstruction and modelling of both purified profiles and cellular ratios, accounting for **tropic variability**.

Keywords: Cellular deconvolution, Tumoral micro environment, Regulatory networks, Transcriptomics, Generative model, Contrained optimisation

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“From *in vitro* to *in vivo*”

From Molecules to Ecosystems: microbial predators in soil ecology”

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Cell Biology of Bacterial Motility Team

Tâm Mignot is a research director in microbiology and head of the Bacterial Chemistry Laboratory, specializing in bacterial cell biology.

Myxococcus xanthus is a predatory bacterium essential to soil ecology. It is able to glide over solid surfaces to feed on other micro-organisms. It is at the heart of Tâm Mignot’s internationally acclaimed work, which includes discovering the molecular mechanism it uses to detect and kill a wide range of prey. Tâm Mignot’s work also sheds light on how M. xanthus manages to move collectively and form multicellular structures resistant to environmental stress. Tâm Mignot’s research has greatly enhanced our understanding of bacterial communities. It has also contributed to the creation of new imaging and artificial intelligence technologies, and to the development of the first commercial microfluidic device adapted to the study of bacterial growth.

Tâm Mignot received the CNRS Silver Medal in 2023 and the CNRS Bronze Medal in 2011.

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"From *in vitro* to *in vivo*" Short-talks

Structure and function of spermatid manchette microtubules

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To perform their cellular functions, microtubules organize into three-dimensional assemblies, within which their dynamics and mechanical properties are tightly regulated. During spermiogenesis, the rigid microtubule-based manchette is crucial for shaping the sperm head and facilitating the transport of materials required for flagellum assembly. The manchette is thus key to generate functional sperm. Despite its importance, the structure and regulatory mechanisms of the manchette microtubules remain poorly studied. In this context, our project aims to characterize an internal structure that we recently discovered within *Xenopus* manchette microtubules by exploring its role in regulating microtubule properties using *in vitro*, *in vivo* and *in situ* approaches.

Proteomics studies and high resolution cryo-electron microscopy uncover the internal organization of *Xenopus laevis* manchette microtubules, revealing the set of proteins forming the structure observed previously. Subsequent studies of these microtubule assemblies *in vitro* will give insight on their dynamic and rigidity properties.

In vivo and *in situ* data on manchette microtubule conservation across species are questioning the role of this structure in spermatogenesis. Our observations reveal its presence in *Xenopus* manchette microtubules and its absence in mammalian ones. While the round spermatid undergoes compaction of its nucleus to form functional spermatozoa, the degree of morphological alteration of sperm head exhibits significant interspecies variation. *Xenopus* manchette microtubules, reinforced with an internal structure, induce the formation of elongated and sharp sperm nucleus, in contrast to the ovoid nuclei of mammalian spermatozoa. Is the internal structure involved in extreme reshaping of spermatid nucleus during spermiogenesis? Moreover, is manchette architecture subjected to adaptive evolution in response to environmental pressures such as viscosity challenges of aquatic fertilization or thermal fluctuation of ectotherm organisms?

The detailed molecular characterization we propose seeks to provide new insights to address sperm morphological abnormalities associated to fertility issues.

Keywords: Microtubule, MIPs, Manchette, Spermatogenesis, Male infertility

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The transcriptional dynamics of muscle progenitors response to Notch signaling

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Notch signaling pathway is conserved across evolution and is involved in a wide range of biological processes, such as myogenesis. Indeed, Notch is implicated in this multi-step process by keeping the muscle progenitors in proliferation and deciphering differentiation entry (Relaix et al., 2005). Recently, it has been shown that variation in Notch activation dynamics could impact the transcriptional responses of its targets (Falo-Sajuan et al., 2019; Lee et al., 2019). Moreover, cell culture experiments have shown that these activity modulations can impact the expression of Notch targets with opposing roles in myogenesis (Nandagopal et al., 2018).
We hypothesize that changes in Notch transcriptional bursting dynamics-including duration, frequency, and amplitude-regulate different target genes, thereby orchestrating muscle progenitors fate decisions.
We implemented the MS2-MCP system to directly measure Notch activity kinetics, in Drosophila muscle progenitors. This system uses MS2 stem-loop sequences inserted into non-coding regions of genes of interest, allowing visualization of nascent transcripts through MCP-GFP accumulation in nuclear puncta (Garcia et al. 2013). We established a new Notch-MS2 transgenic fly line. Using a computational pipeline (Leroux et al., 2023), we demonstrated significant upregulation of the Notch-MS2 sensor upon Notch overexpression, with single-molecule FISH. We optimized imaging protocols for real-time visualization of Notch-MS2 transcriptional dynamics. Upon characterization of transcriptional patterns and their variations, we will proceed with functional studies to elucidate the biological relevance of these dynamics in muscle progenitors fate decisions.
This project aims to provide novel insights into the temporal regulation of gene expression in stem cell biology and its implications for tissue homeostasis and regeneration.

Keywords: Stem cell, Transcription, Notch, Muscle, Development

*Speaker

Molecular mechanisms of aneuploidy and glioblastoma aggressiveness induced by Diaph3 loss

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Diaphanous-related formin 3 (Diaph3) belongs to the formin family of dimeric multidomain proteins that are master regulators of actin and microtubule dynamics. It plays a role in the cytoskeleton remodeling, more specifically in cytokinesis and karyokinesis. In the mouse brain, loss of *Diaph3* induced aneuploidy followed by massive apoptosis in neural stem cell, leading to microcephaly. Given the potential role of aneuploidy in cancer development, we are interested to unravel whether Diaph3 depletion during gliomagenesis could aggravate glioblastoma (GBM) aggressiveness.

To analyze whether Diaph3 is a potential therapeutic target in human GBM, we will use siRNA-mediated depletion of Diaph3 in a panel of GBM cell lines (U251 and U87). Our results showed that siRNA-mediated depletion of Diaph3 in p53 mutated GBM cells (U251 cells) disrupted microtubules and caused mitotic defects such as multipolar mitosis. Karyotyping showed an increase in aneuploidy in Diaph3-depleted U251 cells. Since it has been shown that numerical abnormalities in chromosomes segregation could trigger DNA damage response (DDR), we hypothesize that the loss of Diaph3 induces increased activity of the DDR pathway and we aim to decipher their underlying molecular mechanism.

We believe that this study may provide insights into the mechanisms by which Diaph3 prevents aneuploidy in glioblastoma cells but also, more generally, into the role of aneuploidy in gliomagenesis.

Keywords: Glioblastoma, Aneuploidy, DNA Damage, Gliomagenesis

*Speaker

Kinetochore microtubules flux poleward along fixed centrosome anchored microtubules during the metaphase of *C. elegans* one -cell embryo

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During the cell division, the cell sets up correction mechanisms and checkpoints that allow it to adapt and ensure the fidelity of the division. Poleward flux seems involved in those corrective mechanism, and it consists of a solid movement of the microtubule network towards the centrosome and is especially present in metaphase. This movement is the result of the dynamic instability at each ends of the microtubule, as well as the action of molecular motors allowing the sliding of microtubules among each other. We study this phenomenon using the single-cell *Caenorhabditis elegans* embryo as a model. While all flux involved proteins are present in *C. elegans*, no flux had been detected in this organism. Thanks to technological developments in microscopy and analysis, we conducted FRAP experiments that revealed a poleward flux in metaphase. We found that the flux speed was not constant throughout the spindle, with strong poleward flux close to the metaphasic plate, slowly decreasing as we move further away from the chromosomes. We found that this is due to the fact that only the kinetochore microtubule, which are abundant close to the chromosome, are undergoing poleward flux. In this model, treadmilling does not seems to play a major role in the process, as the depletion of the only *C. elegans* depolymerase, KLP-7MCAK, don't affect the flux speed. This was not the case when we inhibited molecular motors such as kinesin-12 and kinesin-5, respectively KLP-18 and BMK-1 in *C. elegans*, which suggest that the sliding of kinetochore microtubule along the spindle microtubule account for the poleward flux in the *C. elegans* zygote. Those results contrast with the solid like displacement of the microtubule network that has previously been attributed to the poleward flux but are consistent with the length to plate sensitive flux that has been shown in Hela cells. We think that the poleward flux might allow strong cortical pulling forces required for the fast-paced division to occurs while keeping the kinetochores isolated from those forces.

Keywords: *C. elegans*, Spindle, Microtubule, Poleward flux

*Speaker

"From *in vitro* to *in vivo*"
Posters and flash-talk s

From "*in vitro*" to "*in vivo*" - Poster #1

Investigating the Molecular Mechanisms Regulating the Dynamic Sampling of the DNA by the Glycosylase OGG1 and its Ability to Detect 8-Oxoguanine in Living Cells

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Reactive oxygen species (ROS) can induce the formation of numerous DNA lesions, the most abundant being the oxidized form of guanine: 8-oxoguanine (8-oxoG). This lesion has a strong mutagenic potential, promoting G:C to T:A transversion. In human cells, 8-oxoG is handled by the base excision repair (BER) pathway, with the first stage being its detection and excision by the DNA glycosylase OGG1. However, 8-oxoG neither distorts the double helix nor blocks polymerases, implying that OGG1 needs to probe each base pair individually by a mechanism that is still only partially understood. Using fluorescence correlation spectroscopy (FCS) and laser irradiation, I analyzed the dynamics of OGG1 in the nucleus of living human cells. These assays revealed the crucial role played by conserved OGG1 residues in the efficient detection of 8-oxoG within the nucleus. We demonstrated that rapid DNA sampling by OGG1 is regulated by residues N149 and N150, which may maintain the local DNA structure, while residue Y203 probes the double helix in search of 8-oxoG. Our results further suggest that the two arginines, R154 and R204, are essential for the interaction between OGG1 and DNA, as well as for the recognition of the cytosine opposite to 8-oxoG.

Keywords: DNA repair, OGG1, Fluorescence correlation spectroscopy

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Phosphorylation of dBigH1 Facilitates Linker Histone Exchange and Promotes Proper Embryonic Development

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Metazoan genomes usually encode several linker histone variants, often expressed in a tissue- or developmental stage-specific manner. The *Drosophila melanogaster* genome contains only two linker histone variants: H1 is present in somatic cells, while BigH1 substitutes H1 in the germline and early embryos. During embryogenesis, after cellularization, BigH1 is quickly replaced by H1 in the nuclei of somatic cells. However, the molecular mechanism of this exchange and the possible function of post-translational modifications in this process remain poorly understood. Based on proteomics data, 16 potential phosphorylation sites were detected on BigH1. To address the importance of BigH1 phosphorylation, we created mutant *Drosophila* lines with CRISPR/Cas9 mutagenesis, in which the affected serine and threonine amino acids are substituted entirely or partially by alanine in the endogenous BigH1 sequence, thus rendering the synthesized protein incapable of phosphorylation. We found that S265 is a major phosphorylation site, while C-terminal hyperphosphorylation is essential for viability, particularly under suboptimal conditions. Moreover, upon the disruption of phosphorylation sites, an ectopic expression pattern of BigH1 can be detected in late-stage embryos. This phenotype appears to be primarily caused by modifications within the N-terminal domain, as observed via confocal microscopy and Western blot. These results suggest that phosphorylation at T74 and S88 is vital for maintaining the proper expression pattern, possibly either through linker histone exchange or by influencing the turnover rate of the protein. However, salt elution experiments revealed no changes in the binding affinities of the modified proteins. Furthermore, eliminating all potential BigH1 phosphorylation sites reduces overall H1 and H3 levels in late embryos. This phenotype, however, was not observed in the N-terminal mutant, which also displayed prolonged BigH1 expression, suggesting these effects are independent. In summary, BigH1 phosphorylation appears to have a crucial role during early *Drosophila* development and, consequently, in chromatin dynamics.

Keywords: Phosphorylation, dBigH1, Linker histone, Chromatin dynamics

*Speaker

From "*in vitro*" to "*in vivo*" - Poster #3

Microtubule stiffening by ZYG-8 participates in spindle positioning and orientation during *C. elegans* embryo division

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Microtubules, which are semi-rigid and dynamic fibers, are important players of mitosis. Beside constituting the mitotic spindle, an essential structure for correct chromosome segregation, they participate in setting mitotic spindle position, a key element for cell fate determination. While microtubule dynamics are essential during mitosis, the role of their bending-rigidity, namely microtubule capacity to bend under compression, remains unknown. Studies in neuronal cells showed that proteins of the Doublecortin family can regulate microtubule rigidity by binding on microtubules at the vertex of 4 tubulin dimers. We set out to investigate microtubule rigidity's roles during mitosis using the *Caenorhabditis elegans* zygote as an established model of cell division. Interestingly it displays a single member of Doublecortin family: ZYG-8/DCLK1. In the first place, we challenged whether ZYG-8 regulates microtubule rigidity during mitosis. To do so, we segmented microtubules through a semi-supervised machine-learning approach, and evaluated their bending rigidity indirectly by quantifying their longitudinal curvatures and tortuosity (how sinuous the filaments are). Interestingly, in conditions where ZYG-8 binding on microtubules is decreased (*zyg-8(RNAi)*) or inhibited (*zyg-8(or484ts)* mutant), we measured an increase in microtubule local curvatures, tortuosity and curved microtubule proportion, suggesting less rigid microtubules. We then sought functional tasks of ZYG-8 in microtubule bending-rigidity regulation. We recently proposed that microtubule pushing against the cell cortex contributes to positioning the spindle accurately. Interestingly, the pushing force depends on microtubule rigidity. We investigated this positioning and measured, during anaphase, increased spindle-pole-oscillation amplitudes upon *zyg-8(RNAi)*, even stronger with *zyg-8(or484ts)*. The closer proximity of spindle poles to the cell cortex during their oscillations is consistent with reduced cortical pushing forces due to lower microtubule rigidity upon *zyg-8* targeting. Importantly, in mutant embryos, the exaggerated oscillation amplitudes led to strong defects in spindle final positioning and orientation.
Overall, our work reveals that microtubule bending-rigidity participates in spindle positioning and orientation during mitosis, thereby complementing the role of microtubule dynamics. This novel regulation in cell division may also play a role in spindle functioning.

Keywords: Mitosis, Microtubule rigidity, Doublecortin, Spindle positioning, *C. elegans*, Microtubule curvature

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Structural Characterisation of the Donor/Recipient Cell Detection Mechanisms by the Type IV Secretion System

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Bacterial conjugation is a biological process through which a donor cell transfers DNA to a bacterial cell. It is the primary cause of the spread of antibiotic resistance genes among bacterial populations, significantly contributing to the global antibiotic resistance crisis. In Gram negative bacteria, this DNA transfer is mediated by a large molecular machinery – the conjugative type IV secretion system (T4SS) – embedded in the donor cell membranes. To establish the first contact between the two bacteria, the donor cell produces a long extracellular filament - the conjugative pilus – essential for DNA transfer. T4SSs themselves produce an inhibitor to prevent perpetual DNA exchange between two bacteria carrying the same conjugative plasmid. Here, we develop a large-scale native purification protocol to isolate the pilus through their tip. We identify the protein localized at the pilus tip that extends into the extracellular environment. Our finding suggests that the pilus tip interacts with components of the bacterial outer membrane displayed at the surface of recipient cell. We also identify the function, localization and the oligomerization state of the "natural" conjugation inhibitor. This work provides crucial insights, at a molecular level, into the key step of the recipient cell recognition by the pilus tip to potentially be targeted to block bacterial conjugation and thereby limit the spread of antibiotic resistance genes.

Keywords: Antiobiotic resistance, T4SS, Bacterial conjugation, Pilus

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In vitro bioaccessibility method for SVOCs in indoor dust

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Semi-volatile organic compounds (SVOCs) from everyday products can be found in confined spaces and settled dust (Weschler et Nazaroff, 2008). This settled dust can be involuntarily ingested, particularly by young children through hand-to-mouth contact (Anses, 2019). Those compounds present various toxicities and potential endocrine disruption effects. Health risk assessments consider the total fraction of pollutants in ingested dust. However, only the bioaccessible fraction can cross the biological membrane in the gastrointestinal tract and be assimilated by the body to reach target organs. Numerous *in vitro* digestion methods have been developed in recent years to assess bioaccessibility (BA) (Raffy et al., 2018). But most remain complex, costly and do not explore BA for certain organic pollutants of concern in dust. This work provides BA with a simple method for SVOCs in indoor dust for the first time. Food can influence BA for organic compounds and a fed-stated *in vitro* digestion method has been tested. The SVOCs studied were selected according to their occurrence in the indoor environment and their toxicity to humans : phthalates, OPFRs and pyrethroid pesticides.
BA method reproduces *in vitro* digestion with simulated intestinal and colon incubation. Intestinal solution is prepared with bile salts and pancreatin of porcine origin, incorporating an Amberlite™ XAD®-2 sink for SVOCs to mimic continuous absorption of chemicals through the intestinal wall. Tests are carried out using indoor dust SRM® 2585. Dust and sinks are incubated in 30 mL of intestinal solution with 10 mL of reconstituted milks (skimmed, semi-skimmed, whole milks) to reproduce chyme during digestion, at 125 rpm for 20h at 37°C and pH=7. The bioaccessible concentration (BaC) trapped in the sink and the non-bioaccessible concentration (nBaC) left on lyophilized dust were extracted with pressurized liquid extraction and analyzed by GC/MS/MS.

Keywords: *In vitro*, Bioaccessibility, Digestion, Indoor dust, Exposure, Human health, Organic compounds, Pollutants

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Microtubules adopt a new type of lateral interaction in response to excessive protofilament skew

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Departure from the classical 13 protofilaments, 3-start lateral monomer helix architecture, is common in microtubules assembled from purified tubulin, but also occurs in different organisms and cells types. The microtubule lattice accommodates this structural polymorphism by a global skew of the protofilaments in order to preserve the lateral and longitudinal interactions between tubulin subunits, a mechanism geometrically described by the 'microtubule lattice accommodation model'. Here, we analyzed by cryo-electron microscopy microtubules assembled in the presence of GDP-BeF3- and GTPyS, two slowly hydrolysable GTP-analogues. We find that in the presence of these analogues, microtubules can adopt configurations with protofilament skew angles up to 3○, but most of them relax to lower values, likely as a response to tortional stress. Abrupt transitions between stressed and relaxed lattices are occasionally observed within the same microtubules, suggesting a cooperative relaxation process. To visualize this mechanism, we decorated microtubules with kinesin motor domains, analyzed their structure by cryo-electron tomography, and performed segmented sub-tomogram averaging to reveal the underlying tubulin dimer organization within individual microtubules. This analysis revealed long-range dislocations of about 20 Å between two protofilaments, not necessarily at the seams made of heterotypic ab-lateral interactions. We propose that this relaxation mechanism preserves microtubules from torsional stress induced by high protofilament skew, which could otherwise destabilize the microtubule lattice and induce their depolymerization in dynamic assembly conditions, a stochastic event know as catastrophes.

Keywords: Microtubule, Electron microscopy, Structure

*Speaker

From "*in vitro*" to "*in vivo*" - Poster #8

Investigating the DNA binding capacities of the PARP1 BRCT and automodification domains *in cellulo.*

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Each day, our cells experience around 10^5 DNA lesions(1) and maintain their genome integrity through coordinated actions of DNA repair proteins. Among these, the Poly(ADP-ribose) polymerase 1 (PARP1) plays a crucial role in initiating repair mechanisms following recognition of both single and double strand breaks(2,3). PARP1, with the help of its five DNA-binding domains, interacts with DNA until it encounters a lesion, triggering its binding and the subsequent addition of ADP-ribose chains on histones *via* its catalytic domain that signals damage. This post-translational modification found also on PARP1 itself is essential for its release from the break sites(4). PARP inhibitors were designed to prevent this self-modification, inducing a PARP trapping at DNA breaks. Clinical trials have taken advantage of this phenomenon to kill cancer cells(5). However, some patients develop resistance that is probably driven by impaired trapping. Therefore, understanding the precise mechanisms by which PARP1 interacts with DNA breaks is crucial for developing alternative strategies to overcome treatment resistance.
The BRCA1 C Terminus (BRCT) domain of PARP1 has recently been reported as a potent DNA-interacting domain *in vitro* (6). However, evidence of interactions of this BRCT domain and its adjacent auto-modification domain (AD) with nuclear DNA breaks is scarce. Using laser irradiation and fluorescence correlation spectroscopy (FCS), we revealed that the PARP1 BRCT domain mediated the PARP1 interaction with undamaged DNA *in cellulo via* a series of conserved lysine residues and that the AD domain contributed to this interaction even with intact DNA.

References:

(1) T. Lindahl et B. Nyberg, *Biochemistry*, 1972
(2) S. Eustermann *et al.*, *Mol. Cell*, 2015
(3) M.-F. Langelier et al., *Science, 2012*
(4) L. Aberle *et al.*, *Nucleic Acids Res.*, 2020
(5) J. Murai *et al.*, *Cancer Res.*, 2012
(6) J. Rudolph *et al.*, *Mol. Cel,* 2021

Keywords: DNA repair, PARP1, Live cells microscopy

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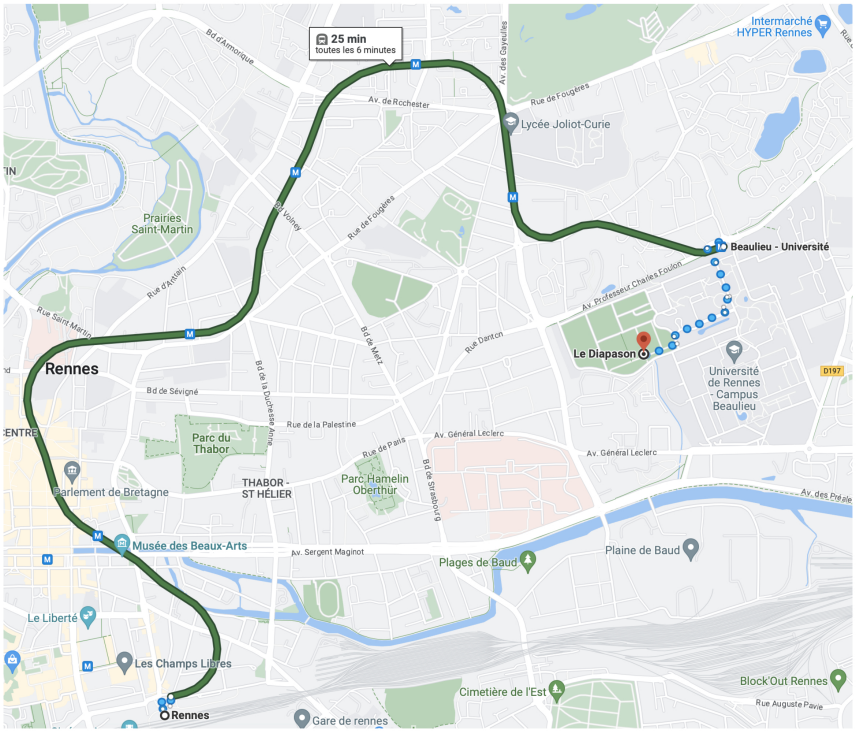

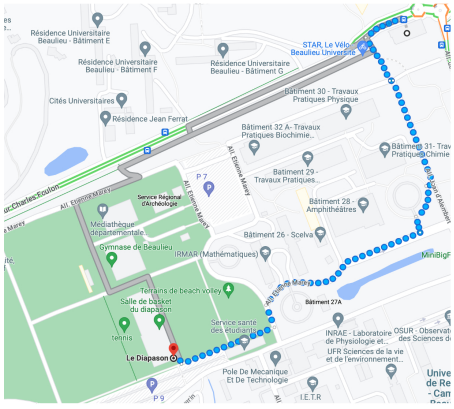

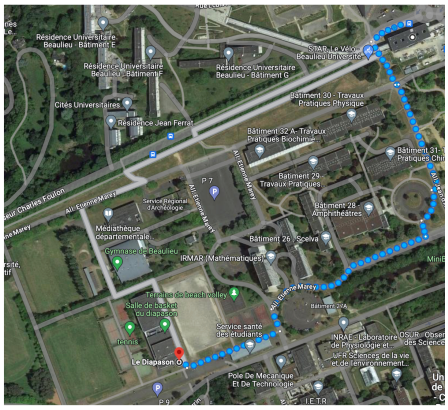

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