Journées de l'Ecole Doctorale de Nice

15th, 16th and 17th of May 2023

Grand Château - Valrose Campus

Avenue Joseph Vallot, 06100 NICE

ABSTRACT BOOK

Guest speakers

Ype Elgersma
Dept Neuroscience, Rotterdam, NL

Michael Clarke
Standford Medecine, USA

Ilse Ariadna Valtierra Gutierrez
Nature Communications Editor

JEDNs 85

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SCIENTIFIC COMMITTEE

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Bruno **Favery**	***Plant biology***

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KEYNOTE LECTURES

This year the JEDNs Committee is thrilled to welcome :

Ype Elgersma, *Dept Neuroscience, Rotterdam, NL*

« Neurodevelopmental disorders: Understanding the mechanisms and identifying treatments »

Neurodevelopmental disorders (i.e. intellectual disability, autism) affect 1% of the population, and often have a genetic basis. Our lab seeks to get insight in the molecular and cellular mechanisms underlying these disorders, with the ultimate goal to develop treatments. Our research is divided into three research lines: (1) Improving genetic diagnosis, (2) Understanding the mechanisms underlying neurodevelopmental disorders (3) Translational studies (i.e. clinical trials) to improve the quality of life of the affected individuals.

Improving diagnosis

To improve genetic diagnosis, we have developed together with van Woerden lab a functional genomics screen (PRiSM) to rapidly determine if a genetic variant is pathogenic. This screen is not only important for providing a diagnosis, but also allows us to get more insight in the genes underlying neurodevelopment.

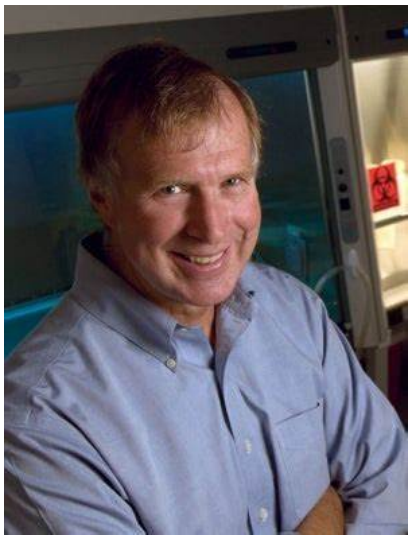
Understanding the mechanisms underlying neurodevelopmental disorders

To get more insight in the pathophysiology of neurodevelopmental disorders, we typically make use of genetically engineered mouse models. Mouse models are analyzed at the biochemical, cellular (electrophysiological) and behavioral level. By analyzing the mouse models at all these levels we try to understand the specific function of these genes and proteins in brain development and learning and memory. Besides mouse models, we are currently also exploring the value of iPS cells to study these disorders. The genes and proteins that we specifically focus on are proteins associated with the RAS-ERK-MTOR signaling pathway and the proteasome.

Translational research

To translate our findings to the patients, we are part of the ENCORE expertise center for neurodevelopmental disorders, for which Ype Elgersma is the scientific director. ENCORE has large expertise outpatient clinic for Angelman Syndrome, Neurofibromatosis (NF1), Tuberous Sclerosis (TSC), Fragile X and genetic forms of autism.

Michael F. Clarke, *Standford Medecine*, USA



« Stem Cell Biology and Regenerative Medicine »

In addition to his clinical duties in the division of Oncology, Dr. Clarke maintains a laboratory focused on two areas of research: i) the control of self-renewal of normal stem cells and their malignant counterparts; and ii) the identification and characterization of cancer stem cells. A central issue in stem cell biology is to understand the mechanisms that regulate self-renewal of hematopoietic stem cells, which are required for hematopoiesis to persist for the lifetime of the animal. Until recently, the molecular mechanisms that regulate adult stem cell self-renewal were not known. His laboratory recently found that the proto-oncogene Bmi-1 regulates stem cell self-renewal via an epigenetic mechanism. By investigating the pathways upstream and downstream of Bmi1, the laboratory is actively investigating the molecular pathways that regulate self-renewal.

Cancers arise as a result of a series of genetic mutations. A better understanding of the consequences of these mutations on the underlying biology of the neoplastic cells will help to focus the development of more effective therapies. Solid tumors such as breast cancers contain heterogeneous populations of neoplastic cells. Dr. Clarke□s group has developed a technique that allows the isolation and characterization of tumorigenic and non-tumorigenic populations of cancer cells present in human breast, colon and head and neck cancer tumors. Only a small minority of cancer cells had the capacity to form new tumors in a xenograft model. This tumorigenic cell population could be identified prospectively and consistently had definable and identical phenotype. The tumorigenic cells displayed stem cell-like properties in that they were capable of generating new tumors containing additional stem cells as well as regenerating the phenotypically mixed populations of non-tumorigenic cells present in the original tumor. Effective treatment of cancer will require therapeutic strategies that are able to target and eliminate this tumorigenic subset of cells. The laboratory is pursuing the identification of cancer stem cells in other tumors so that they can be studied. Dr. Clarke□s laboratory will provide other members of the program with the expertise to identify and isolate cancer stem cells from solid tumors of epithelial origin. Finally, the laboratory is actively pursuing how cancer stem cells self-renew to maintain themselves and escape the genetic constraints on unlimited self-renewal that regulate normal stem cell numbers. Differences in self-renewal pathways between normal and malignant stem cells could be targeted by new therapeutic agents to eliminate cancer stem cells.

PROGRAM

MONDAY 15th of MAY

Oral Communications

8:30	REGISTRATION
9:00	OPENING
9:30	EUR LIFE BGI DUTSCHER
10:00	**KEYNOTE LECTURE** **M. CLARKE**, Stanford, USA *Stem Cell Biology and Regenerative Medicine*
10:45	Coffee Break
11:15	THERMOFISCHER
11:30	**3rd YEAR PhD TALKS** 1 – *Cancer* – **E. PAPASOTIRIOU** – C3M 2 – *Bioinfo* – **A. COLLIN** – IPMC 3 – *Plant Bio* – **M. COINTE** – ISA 4 – *Neurobio* – **M. VILLET** – IPMC
12:30	**2nd YEAR PhD POSTERS TEASING 1**
1:00	Lunch Break
2:00	**3rd YEAR PhD TALKS** 5 – *Biochem / Physio* – **P.L. BATROW** – iBV 6 – *System bio* – **M. BARTHE** – IPMC 7 – *Dev bio* – **A.B. TANARI** – iBV 8 – *Physio* – **L. CLOTAIRE** – LP2M
3:00	**2nd YEAR PhD POSTERS SESSION** / Coffee Break
4:00 6:00	**3rd YEAR PhD TALKS** 9 – *Cancer* – **C. GOTORBE** – CSM 10 – *Immuno* – **M. BOURINET** – C3M 11 – *Dev bio / Marine bio* – **A. VULLIEN** – IRCAN 12 – *Biochem / Physio* – **N. RACHEDI** – IPMC 13 – *Cancer / System bio* – **A. CHALABI** – IPMC 14 – *Immuno* – **S. BENHMAMMOUCH CARMINO** – C3M 15 – *Dev Bio / Neuro* – **N. FJERDINGSTAD** – iBV

MONDAY 15th of MAY

Poster Session

1	*Neurobiology*	**BOULSKIBAT Asma**	IPMC
2	*Physio - Electrophy*	**VIVIER Charles-Maxime**	IRCAN
3	*Plant bio*	**LUPATELLI Carlotta**	ISA
4	*Developmental bio*	**VALERO Florian**	iBV
5	*Cancer*	**DURANDY Manon**	IRCAN
6	*Biochemistry*	**ANGELELLI Francesco**	C3M
7	*Immuno*	**TEISSEYRE Maxime**	UR2CA
8	*Neurobiology*	**VANDERSTEEN Clair**	CoBTeK
9	*Bioinformatics*	**OKOROKOVA Larisa**	IRCAN
10	*Immuno / Physio*	**MUCEL Inès**	C3M
11	*System biology*	**MARTINEZ SANCHO Lou**	RETINES
12	*Cancer*	**BOUVET Océane**	C3M
13	*Developmental bio*	**DETTI Mélanie**	iBV
14	*Immuno / Microbio*	**FICHANT Arnaud**	ISA
15	*Physio - Electrophy / Neuro*	**SIMONTI Martina**	IPMC
16	*Cancer / Immuno*	**MOUSSET Alexandra**	IRCAN
17	*Physio - Electrophy*	**TOFT Maurizio**	IPMC
18	*Microbiology*	**PLUMB Emily**	iBV
19	*Biochemistry / neuro*	**MINNITI Julien**	IPMC

TUESDAY 16th of MAY

Oral Communications

8 :30	REGISTRATION
9 :00	AJC06 NEB
9 :45	**3rd YEAR PhD TALKS** 16 – *Cancer* – **Y. GUO** – C3M 17 – *Bioinfo / Omics* – **V. BILLON** – IRCAN 18 – *Cancer/Immuno* – **A. KRUG** – C3M
10 :30	Coffee Break
11 :00	**3rd YEAR PhD TALKS** 19 – *Cancer* – **C. PISIBON** – C3M 20 – *Physio* – **J. DUNOT** – IPMC
11 :30	PROTEINTECH
11 :45	**2nd YEAR PhD POSTERS TEASING 2**
12 :15	Lunch Break
1 :30	**KEYNOTE LECTURE** **Y. ELGERSMA**, Rotterdam, NL *Neurodevelopmental disorders: Understanding the mechanisms and identifying treatments*
2 :15	**3rd YEAR PhD TALKS** 21 – *Cancer* – **V. NICOLINI** – IRCAN 22 – *Cancer / Dev bio* – **A. FARNET** – iBV
3 :00	**2nd YEAR PhD POSTERS SESSION**
4 :00	**3rd YEAR PhD TALKS** 23 – *Biochem* – **H. SAFWAN ZAITER** – iBV 24 – *Bio modeling* – **C. GUICHARNAUD** – ISA
4 :45	Coffee Break
5 :15	**3rd YEAR PhD TALKS** 25 – *Cancer* – **M. TEISSEIRE** – IRCAN 26 – *Immuno* – **B. DOLFI** – LP2M 27 – *Neurobio* – **J. CANAGUIER** – IPMC
6 :45 00 :00	**EVENING EVENT**

TUESDAY 16th of MAY

Poster Session

20	*Immuno*	**CHAFIK Abderrahman**	C3M
21	*Cancer*	**KUNZ Sarah**	IRCAN
22	*Physio - Electrophy*	**CONTU Laura**	iBV
23	*Neurobiology*	**ROYON Léa**	IPMC
24	*Plant bio*	**NJEKETE Cliven**	ISA
25	*Microbiology*	**ORTIS Morgane**	Micoralis
26	*Cancer / Physio*	**TROJANI Marie-Charlotte**	TIRO/MA
27	*Neurobiology*	**FREMONT Gwendoline**	IPMC
28	*Physio - Electrophy*	**BIED Marion**	iBV
29	*Plant bio / Omics*	**GOODLUCK Benjamin**	ISA
30	*Neurobiology*	**LANDES-CHÂTEAU Cassandre**	UR2CA
31	*Developmental bio / Marine bio*	**DESHURAUD Romane**	IRCAN
32	*Immuno*	**ZAIR Fairouz**	LP2M
33	*Neurobiology*	**CŒUR Estelle**	CoBTeK
34	*Physio - Electrophy / Neuro*	**GILBERT Nicolas**	IPMC
35	*System biology*	**LIN Peipei**	IRCAN
36	*Cancer / Immuno*	**KERRENEUR Emeline**	C3M
37	*Neurobiology*	**SOLYGA Mathilde**	iBV
38	*Structural bio*	**OUERTANI Sarah Amira**	iBV

WEDNESDAY 17th of MAY

Oral Communications

8 :30	REGISTRATION
	TEBUBIO
8 :45	**3rd YEAR PhD TALKS** 28 – *Cancer / Immuno* – **R. ELALDI** – IPMC 29 – *Dev bio / Physio* – **C. HERAULT** – iBV 30 – *Neurobio* – **A. PLONKA** – CoBTeK
9 :30	PhD STUDENTS WELL-BEING
9 :45	**3rd YEAR PhD TALKS** 31 – *Biochem* – **N. AL-QATABI** – IPMC 32 – *Cancer* – **Z. SERVANT –** iBV 33 – *Plant bio* – **S. SOULE** – ISA
10 :30	Coffee Break
11 :00	**KEYNOTE LECTURE** **I. VALTIERRA GUTIERREZ**, Nature Communications *Q&A session about scientific editing*
11 :45	**2nd YEAR PhD POSTERS TEASING 3**
12 :15	Lunch Break
1 :30	**3rd YEAR PhD TALKS** 34 – *Physio* – **E. REDMAN** – IPMC 35 – *Cancer / Physio* – **C. CLOT –** iBV
2 :00	**2nd YEAR PhD POSTERS SESSION**
3 :00	**ROUND TABLE** **M. CLARKE**, Stanford, USA **I. VALTIERRA GUTIERREZ**, Nature Communications **K. JENOVAI & A. MASSIAS**, Syneos Health *Discussion about the after thesis and the different professional options available after a thesis*
4 :00	**AWARD & CLOSING CEREMONY**

WEDNESDAY 17th of MAY

Poster Session

39	*Neurobiology*	**CHATO ASTRAIN Isabel**	IPMC
40	*Biochemistry*	**SALAME Sarah**	IPMC
41	*Marine bio*	**ANDREONI Rita**	IRCAN
42	*Immuno*	**MOSKALEVSKA Iryna**	IRCAN
43	*Developmental bio / Neuro*	**DALLORTO Eleonora**	iBV
44	*Plant bio / Microbio*	**NAZARET Fanny**	ISA
45	*Physio - Electrophy*	**KAYATEKIN Mete**	LP2M
46	*Neurobiology*	**BADOT Céline**	IPMC
47	*Immuno*	**BELDI Ghada**	LP2M
48	*Physio - Electrophy / Plant bio*	**RANTY-ROBY Sarah**	ISA
49	*Developmental bio / Neuro*	**PIOVANI Paolo**	iBV
50	*Cancer / Immuno*	**PIERANTONI Alessandra**	IRCAN
51	*Bio modeling*	**WINTER Rémy**	UR2CA
52	*Biochemistry / Physio*	**LEGROUX Ilona**	IPMC
53	*Immuno / Microbio*	**DURAND Tristan**	other
54	*System biology*	**MIRA Thierry**	RETINES
55	*Cancer*	**KAHI Michel**	C3M
56	*Immuno / Microbio*	**AIEM Elody**	Micoralis
57	*Neurobiology / Structural bio*	**KALBFEIS DIT DARNAS Aurélien**	iBV

ORAL COMMUNICATIONS

3rd year PhD Students

OC-1

Cancer	**PAPASOTIRIOU Evangelia**	C3M

Identification of actionable vulnerabilities in the metastatic uveal melanoma

Uveal melanoma (UM) is a lethal ocular cancer. It is the most frequent primary ocular malignancy in adults. Unfortunately 50% of the patients develop metastasis, which are extremely refractory to existing therapeutic treatments. Recently, tebentafusp, an immunotherapy, showed for the first time a significant increase in the overall survival of the patients. This treatment is restricted to patients with specific HLA type (HLA-A*02 :01), a phenotype found only in 9% of the patients. After the diagnosis of the metastatis, 80% of the patients die within a year. The current therapeutic options do not meet the needs of the patients and therefore there is an urgent necessity to reveal the molecular mechanisms of the progression of UM and discover therapeutic targets.

I conducted a genetic CRISPR-Cas9 screening using a kinase bank targeting the total kinome of human metastatic uveal melanoma cells with GNAQQ209Pmutation, a main alteration found in humans. The cells were isolated from hepatic metastasis, the primary site of UM dissemination.

The genetic screening has highlighted as a top-hit a kinase implicated in the maturation of transfer RNA (tRNA). The expression of this kinase has been associated with shorter overall survival in humans (TCGA), strengthening the notion that it is a good molecule- candidate. My results show that inhibition of this kinase decreases the number of mUM cells accompanied by p21 increase, the gatekeeper of cell cycle. Therefore, this kinase is a key player for the proliferation of mUM cells.

According to its aforementioned role in translational fidelity, I investigated the importance of this kinase in the global protein synthesis. My results show that protein biosynthesis de novo is significantly lower after the inhibition of my kinase of interest compared to the control condition.

Lastly, to understand deeper the mechanisms underlying kinase's activity in mUM cells, I did proteomics and transcriptomics. The analysis of transcriptomic results exhibited an enrichment of genes implicated in the interaction of receptors and Extracellular matrix (ECM). These results will be combined with the data from proteomics. The new downstream targets, that will be identified, will actually be new therapeutic targets non-studied so far for the disease of mUM. These targets will be validated both in vivo and in vitro. Furthermore, these candidates could represent new biomarkers for the prognosis of mUM. Mainly they represent promising therapeutic targets to halt the progression of UM, opening new pathways from fundamental science to clinical research.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-2

Bioinformatics **COLLIN Antoine** IPMC

Building a single-cell atlas of the COPD lung

Single cell technologies are transforming biology by allowing a quantitative expression of large numbers of biomolecules at a cellular resolution. Pascal Barbry's group works on these approaches since 2015 and contributes since 2018 to the international Human Cell Atlas consortium that aims at building a comprehensive atlas of the 1013 cells present in a human body.

We first established a human healthy airway cell atlas (Deprez et al., 2020), which was then integrated in a larger Human Lung Cell Atlas (HLCA) dataset that combines 584,000 cells derived from healthy individuals (Sikkema et al., 2022). HLCA captures the cellular diversity of the human lung and describes the characteristics of 62 distinct cell types under different contexts (age, BMI, smoking status).

We are now comparing directly 10 patients suffering from early stages of Chronic Obstructive Pulmonary Disease (COPD) with 12 healthy age-matched volunteers. This dataset of more than 400 000 cells, collected from brushings or biopsies made between the nose to the 6th division of the airways, results from the integration of 119 independent samples. This results in the first COPD airway cell atlas.

Our data reveals significant variations of gene expression between identical cell types from the nasopharyngeal and tracheobronchial airways and also improved the description of rare cells. We have identified altered proportions in some specific cell types at early-stage COPD, such as mucus-producing cells or basal cells, and documented cell-type specific changes in gene expression. Mapping of cells from our COPD atlas onto HLCA improves cell typing and better defines the driving modifications in gene expression during early COPD. The benefits of integrating healthy and disease single-cell atlas in larger resources leads to a more robust characterization and better recovery of cell types and states. This finally establishes the global HLCA as a valuable resource for future investigations and defines a general computational strategy transferrable to the analysis of other pathological samples.

The handling of this particularly large dataset has required to develop a specific pipeline in order to reduce the different batch effects. The resulting resource provides a high-resolution map of lung in normal and pathological settings. We are now identifying compositional shifts and cell type modifications induced by the pathologies that will be confirmed by spatial transcriptomics approaches that we are currently developing.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-3

Plant biology **COINTE Mélina** ISA

From the laboratory to the field, from movement to dispersal: a study of the spread of parasitoids of the genus Trichogramma at different spatial and temporal scales

Dispersal, i.e., the spatial spread of individuals (dispersal sensu lato) may have consequences on reproduction and gene flow (dispersal sensu stricto). It is a subject of paramount importance in ecology since it affects not only individual fitness but also population genetics, population dynamics, and ultimately species distribution. It is also mentioned as a key element in the quality of biocontrol agents. Yet, it remains difficult to measure, in particular because organisms used in biocontrol are often very small. This is notably the case for one of the most used worldwide, the Trichogramma, a parasitoid micro-wasp, measuring less than 1mm, that we used as a study organism. Classically, their dispersal is studied using field releases, but these are time-consuming, expensive and the results are very variable, preventing high-throughput and repeatability. It can also be evaluated using small-scale behavioural trials. However, these experiments neglect important larger-scale processes such as group dynamics. As a result, the correct assessment of dispersal parameters is often complicated and insufficient in breeding programmes for biocontrol or in behavioural studies more generally. To get a step further, we studied the movement and dispersal of 17 strains of Trichogramma cacoeciae at three different scales. First, from experiments in a small experimental arena (a rectangular arena with the following dimensions 11x14.5cm), we computed a proxy of dispersal from the average speed of individuals, their activity rate and the sinuosity of their paths. In a larger scale, a double spiral arena offering a 5.75m long pathway, approaching field dimensions, we obtained a direct measure of the rate of spatial spread (Mean Squared Displacement and diffusion coefficients) of groups of Trichogramma. Finally, we indirectly measured dispersal in field conditions by releasing Trichogramma individuals in rows of bell pepper in greenhouses. The metrics of dispersal obtained at the three scales were then compared to one another. We discuss the extent to which it is possible to extrapolate dispersal metrics across scales, and the implications of this for biocontrol.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-4

Neurobiology **VILLET Maxime** IPMC

Disengagement of the prefrontal cortex in the automation of working memory

Villet Maxime1, Marie Hélène1, Bethus Ingrid1

1Université Côte d'Azur, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, France

Working memory refers to the temporary representation of information that has just been experienced or retrieved from long-term memory. These representations are of short duration but can be maintained for longer periods. They also are subject to various operations that make the information useful for goal-directed behavior. Several studies in the field have identified the prefrontal cortex (PFC) as one of the main brain regions implicated. Indeed, inhibition and recording experiments have shown that this structure is fundamental for working memory (Bauer et al, 1976; Miller et al, 1996) and involved in the information encoding of this memory in association with the hippocampus (Spellman et al, 2015). Our study focuses on the role of the PFC in the formation of the spatial working memory (SWM). To decipher the role of the PFC, we used an inducible inhibition technique (DREADD) while we assessed SWM with a delayed non-matching to place task in a T-maze. Surprisingly, when performing this task, we saw an automation of the behavior once the mice have learn that could be due to a relay structure of the PFC in the SWM. As striatum is known to be the main brain region implicated in automation behavior (Killcross et al,.2003), we decided to make an inhibition of the PFC and the striatum while performing the same task of delay non-match to place. This approach allows us to show the essential involvement of PFC during the learning phase of the SWM while it becomes non-essential when automation take place with the striatum taking the lead.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-5

5 *Biochemistry / Physio* **BATROW Pierre-Louis** iBV

Effect of PPARα deletion specifically in brown adipocytes on adipocytes biology

Batrow P-L1, Gautier N1, Rochet N1, Rekima S1, Maret M2, Mothe-Satney I1 and Amri EZ1
1Université Côte d'Azur, CNRS, Inserm, iBV, Nice, France
2Université Cote d'Azur, INSERM, CNRS, IRCAN, 06107 Nice, France

Introduction: White adipose tissue (WAT) is composed of adipocytes with a single, large lipid droplet and few mitochondria. Their function is to store and release energy in the form of lipids. Conversely, adipocytes located in brown adipose tissue (BAT) possess multilocular lipid droplets, high mitochondrial density, and express uncoupling protein 1 (UCP1) that dissipates excess energy in the form of heat.

Thermogenic adipose tissue is present and active in healthy human adults. Therapeutics aiming at activating this tissue represent a great opportunity to treat obesity and metabolic disorders. Peroxisome Proliferator-Activated Receptor (PPAR) family is known to contribute to this browning process and is involved in fatty acid oxidation and adipogenesis. The study of the three PPAR isoforms (α, β and γ) is complicated by the fact that they share target genes and have redundant functions. The role of PPARα is well studied in the liver but not yet in BAT, although its expression is high.

Methods: The present study aims to determine the role of PPARα in thermogenic function by using BAT specific PPARα knockout mice (PparaBATKO) fed either a chow or a high-fat diet (HFD) and challenged or not with CL316,243 (β3 adrenergic receptor agonist). Gene expression of adipogenic, lipogenic, thermogenic markers was evaluated by RT-PCR and western-blot in BAT and subcutaneous WAT. The morphology of these tissues was analyzed. Targeted lipidomic analysis was performed in BAT.

Results: We observe changes in AT volume and fat pad weight, and an increase in BAT lipid droplet size in PparaBATKO mice. In response to β3-adrenergic stimulation, PparaBATKO mice on HFD display: 1) a modification in BAT lipid content; 2) a decrease in thermogenic marker expression (UCP1, CPT1M, PGC1α) and 3) an increase in the expression of lipogenesis enzymes (ChREBPβ, ACLY, ACC1, FASN, SCD1). BAT oxylipin profile and fatty acid content in triglycerides are also modified in PparaBATKO mice on HFD after β3-adrenergic stimulation. Arachidonic acid and other PUFAs are less abundant in triglycerides while some oxylipins synthesis (cox2 dependent and 12-HETE) is blunted in PparaBATKO mice on HFD especially after β3-adrenergic stimulation.

Conclusion: Our data demonstrate a role for PPARα in BAT thermogenesis and show that PPARα could act as a regulator for ChREBPβ-mediated lipogenesis, affecting BAT lipid homeostasis.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-6

System biology **BARTHE Manon** IPMC

Development and Validation of an In Vitro Human Skin Model to Mimic Epidermal Barrier Damage

Manon Barthe1,2 , Laurie Perdigon1 , Véronique M. Braud2 , Jean-Paul Thénot1 & Hanan OsmanPonchet1 1PKDERM Laboratories, Grasse Biotech, 45 Bd Marcel Pagnol, 06130 Grasse - France 2Université Côte d'Azur, CNRS UMR7275, Institut de Pharmacologie Moléculaire et Cellulaire - France

The poster presents the development and validation of an innovative in vitro human skin model designed to mimic epidermal barrier damage. The model enables the testing of molecules or products for the prevention or repair of damaged skin. The study utilized both chemical and physical methods, including sodium dodecyl sulfate (SDS), tape stripping, and Ambu® Skin Pad, to induce epidermal barrier damage in ex vivo human skin samples. The results of the study demonstrated that SDS treatment led to a decrease in TransEpithelial Electrical Resistance (TEER), while tape stripping and Ambu® Skin Pad treatments increased TransEpidermal Water Loss (TEWL). At the transcriptomic level, SDS and Ambu® Skin Pad treatments caused an upregulation of interleukin-8 (IL-8) mRNA expression and a downregulation of Filaggrin and Loricrin mRNA expression. Moreover, all three treatments induced a decrease in stratum corneum thickness and an increase in dermal absorption of lucifer yellow, with Ambu® Skin Pad treatment being the most effective compared to SDS and tape stripping. Overall, these results clearly indicate a compromised skin barrier. The development and validation of this in vitro skin model of damaged skin offers a valuable tool for testing products that prevent or repair damaged skin, as well as for studying the mechanisms involved in the development and repair of the epidermal barrier. In conclusion, the study lays the groundwork for future research in this field, leading to improved treatments and a better understanding of the fundamental mechanisms involved in maintaining healthy skin.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-7

Developmental bio | **TANARI Abdul Basith** | iBV

Tuning cytoskeletal and mechanical polarity from the cell to the embryo scale to trigger gastrulation movements

During embryo development, tissues remodel their shape under the action of biomechanical forces. Contractile networks of F-actin and non-muscle myosin II (MyoII) constitute a primary force-generating machinery in epithelial cells. Embryo-scale polarized force patterns are necessary to initiate coordinated epithelial movements and shape changes. How actomyosin cytoskeleton polarity is tuned at the cell scale to ultimately result in the emergence of embryoscale polarized force patterns is still poorly understood. To investigate this, we use the early developing Drosophila model system. During the blastula-to-gastrula transition (i.e., during end of cellularization), the F-actin network and the MyoII distribution is spatio-temporally remodeled and tuned at both the basal and apical sides of epithelial cells establishing a polarized pattern along the embryo dorsal-ventral axis1 . For instance, basal MyoII accumulation in ventral cells rapidly vanishes to then reappear apically. This eventually results in a polarized force field driving tissue coordinated movements initiating embryo gastrulation. Here we investigate the cellular mechanisms responsible for fine tuning the F-actin network and the MyoII distribution at basal and apical cell sides. In addition, we investigate how these mechanisms are regulated with high spatio-temporal specificity across the embryo. Finally, by employing advanced light sheet imaging, quantitative live image analysis, optogenetics, and laser manipulation, our research will shed new light on the mechanisms and regulatory factors driving actomyosin polarity from the cell to the embryo scale, ultimately initiating embryo gastrulation.

1 Rauzi, M. et al. Embryo-scale tissue mechanics during Drosophila gastrulation movements. Nat Commun 6, 8677, doi:10.1038/ncomms9677 (2015).

ORAL COMMUNICATIONS

3rd year PhD Students

OC-8

Physio - Electrophy **CLOTAIRE Laetitia** LP2M

Therapeutic strategy to compensate pyrophosphate deficiency in Pseudoxanthoma Elasticum

Laetitia Clotaire [1,2], Isabelle Rubera [1], Nastassia Navasiolava [3], Saïd Bendahhou [1], Christophe Duranton [1], Ludovic Martin[3] and Georges Leftheriotis [4]

Pseudoxanthoma elasticum (PXE; OMIM264800) is a rare inherited disease characterized by progressive ectopic calcification most affecting skin, eyes, arteries, and kidney. This disease is caused by biallelic mutations of *ABCC6* gene (400 variants) which encodes an ATP-binding cassette (ABC) transporter primarily found in hepatocytes and renal tubular cells. Calcification in PXE is associated to low plasma circulatory pyrophosphate level ($[PPi]_{pl}$), a major inhibitor of soft tissue calcification.

We have explored the pathophysiological relationship between $[PPi]_{pl}$, gender, age, disease severity and pathogenic ABCC6 variants. We analyzed a cohort of 44 PXE patients with biallelic ABCC6 variants from the French-based PXE reference center in Angers. We identified 41 distinct ABCC6 variants: Nonsense variants were most frequent (45.5%), homozygous missense (29.5%), deletion (18.2%) and other variants mutations (6.8%) including splicing, frameshift, inversion, and insertion. R1141X variant was most frequent among the 41 variants (39%). Independently of ABCC6 genotype, our data confirmed a decrease (>51%) in $[PPi]_{pl}$ in PXE patients (0.74 (0.33) µmol/L, median (Iqr)), compared to non PXE-population (1.54 (0.46) µmol/L, n=26, $p<0.0001$). Remarkably, affected females showed higher PPi levels than males. A strong correlation between age and PPi in PXE patients suggests that time (i.e., age) appears to be the major determinant of disease severity in PXE. Although, $[PPi]_{pl}$ may provide a biological clue for the clinical diagnosis, our data shed light on the complexity of PPi homeostasis and its role on calcification. This highlights the need to increase the plasma level of PPi to limit low [PPi]pl exposure.

In this context, we are investigating PPi supplementation on PXE patients to normalize their circulatory PPi level by performing a phase II randomized placebo-controlled clinical trial (PROPHECI; NCT04868578). In this interventional trial, we will evaluate the safety and efficacy of a daily oral administration of PPi salts. The trial includes two PXE arms of study. 66/99 patients supplemented with 40mg/kg of PPi salts and 33/99 patients with placebo for one year. Our trial is an important step to understand the oral pharmacokinetic profile of PPi and elucidate the potential protective effect of PPi in PXE patients.

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ORAL COMMUNICATIONS

3rd year PhD Students

OC-9

Cancer | **GOTORBE Célia** | CSM

INHIBITION OF WARBURG EFFECT DISRUPTS INTRINSIC RESISTANCE TO FERROPTOSIS OF THE COLON ADENOCARCINOMA CELLS

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Ferroptosis is a newly described reactive oxygen species (ROS)-dependent type of regulated cell death. The major event leading to ferroptosis is free iron-catalyzed oxidation of the lipids in the plasma membrane, resulting in the disruption of its integrity, cell bubbling, and finally death by explosion. This type of cell death is mainly prevented by glutathione peroxidase 4 (GPX4) that uses glutathione (GSH) as a reducing power. Furthermore, recently an alternative pathway consisting of reduced-ubiquinol and its regenerating enzyme - ferroptosis suppressor protein 1 (FSP1), has been described. This cell death can also be pharmacologically prevented using lipophilic antioxidants such as vitamin E or ferrostatin-1.

The potential of ferroptosis as a powerful anticancer strategy has been widely recognized. However, still very little is known regarding the resistance mechanisms of some cancer cells to this type of cell death. To investigate this issue, we used colon adenocarcinoma (CRC) cell lines that, according to our data, showed superior resistance to ferroptosis induced either by genetic invalidation of FSP1 alone or in combination with inhibition of GPx4 by RSL3.

To sensitize these cells to ferroptosis, we then generated glycolysis-null cells (cells with genetically deleted both isoforms of lactate dehydrogenase, LDHA and LDHB) in order to increase the cellular ROS level by switching to oxidative metabolism (oxidative phosphorylation, OXPHOS). And indeed, the sensitivity to inhibition of both anti-ferroptotic axes (GPx4 and FSP1) was fully revealed in these "Warburg effect-incompetent" cells, showing typical features of ferroptosis, including lipid hydroperoxide accumulation, bubbling and ferrostatin-preventable cell death.

In conclusion, our data indicate that anti-ferroptotic pathways of some cancer cells operate in the overall physiological context and in some instances their inhibition should be coupled with other metabolic modulators.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-10

Immuno	**BOURINET Manon**	C3M

CD44-mediated regulation of liver and intestinal group 1 innate lymphoid cells impacts the development and progression of metabolic steatohepatitis

Obesity is one of the major public health problems in developed countries and its incidence is constantly increasing. In addition to impairing quality of life, obesity increases the risk of developing type 2 diabetes and cardiovascular disease. Obesity is also accompanied by frequent but underestimated liver complications, due to their slow evolution. These abnormalities are due to an accumulation of fats in the liver ("fatty liver" or steatosis) which eventually leads to inflammation (steatohepatitis) and can evolve into cirrhosis and cancer. It is therefore urgent to understand the precise mechanisms that lead to this inflammation in the liver in order to propose therapeutic targets to patients affected by this pathology. We are interested in a family of immune cells, the natural killer cells (NK cells), which are known to regulate the mechanisms of inflammation in different tissues of the body including the liver. We hypothesized that dysfunctions in these cells could contribute to the development of liver diseases. Our results have already shown in a model of liver complications that the absence of the CD44 in these immune cells aggravates the disease (liver injury, inflammation, fibrosis). These data suggest that this molecule regulates the functions of these cells. Our current goals are to better understand how these immune cell functions are controlled during the development and progression of liver complications. This project aims to propose new therapeutic and diagnostic approaches to patients suffering from these obesity-related liver diseases.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-11

Dev bio / Marine bio **VULLIEN Aurore** IRCAN

Comparative analysis of the involvement ROS at the onset of regeneration in Platynereis dumerilii and Nematostella vectensis

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Injury-induced regeneration is a common and widespread process in animals. Although it is limited to a few tissues in humans, many species are able to regenerate limbs or even their entire body (Tiozzo & Copley 2015). Fundamental questions about the cellular and molecular basis of animal regeneration remain unanswered and it is still largely unknown if regeneration may rely on universal mechanisms. Identifying conserved or specific cellular and molecular mechanisms in different models, potentially pro-regenerative ones, could contribute to answer these questions and offer new medical perspectives (Grillo et al 2016). A few recurring mechanisms have been identified during regeneration in several models. In these species, apoptosis is a key event, triggering compensatory proliferation in neighbouring cells (Perez-Garijo & Steller 2015). Cell death during regeneration in several species is stimulated by production of reactive oxygen species (ROS) by cells of the wound epithelium. ROS have been shown to be essential for regeneration in several models and to act through the activation of MAPK and/or JNK pathways (Vriz et al 2014). However, whether the "ROS → apoptosis → proliferation" cascade may represent a general principle of regeneration and involve conserved signaling pathways is not known.

My research project aims at deciphering the role of ROS during regeneration in two evolutionarily distant animals, the cnidarian Nematostella vectensis and the annelid Platynereis dumerilii, both displaying extensive regenerative capacities. I used genome screening and molecular phylogeny to identify ROS metabolism genes in both species and explore their evolutive history among metazoans. I investigated their spatio-temporal expression patterns during regeneration using RNAseq data and in situ hybridization and found that most of them are dynamically expressed during regeneration, with up-regulations as well as spatial patterns of pro-oxidative and anti-oxidative genes overlapping. Labelling experiments using ROS-sensitive dyes show that ROS are produced at the amputation site, preceding known events of cell death and proliferation. Finally, I performed functional analyses using ROS inhibitors to test the requirement of ROS production for successful regeneration and found that such treatment delayed or stalled regeneration in both models. Further characterization showed that ROS inhibition particularly affects proliferation in P. dumerilii.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-12

Biochemistry / Physio **RACHEDI Nesrine** IPMC

Extracellular matrix remodeling and its implication in pulmonary vascular cells reprogramming during pulmonary arterial hypertension

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Pulmonary Arterial Hypertension (PAH) is a deadly and enigmatic pulmonary vascular disease characterized by an intense remodeling of the pulmonary arterial wall. This remodeling is driven by an excessive proliferation of multiple vascular cell types, leading to increased pulmonary vascular resistance, and ultimately, to severe heart failure. There exist over a dozen approved vasodilatory therapies which slow down PAH progression, however, none of them prevents it or cures it by targeting its molecular origins. The extracellular matrix (ECM) is the main extracellular component of tissues and organs. It's a dynamic structure that provides a physical support to cells and regulates intercellular biochemical and biomechanical signaling. Perturbations of this network result in a loss of cell and tissue homeostasis and lead to several diseases. Recently, we reported that activation of resident adventitial fibroblasts drives ECM remodeling to promote pulmonary vascular dysfunction in PAH. Yet, activated fibroblasts produce and secrete hundreds of ECM proteins. Whether and how they affect vascular wall structure and reprogram vascular cells to promote PAH remain unknown. Here, using a combination of transdisciplinary approaches including primary vascular cells co-culture, confocal microscopy, proteomics, metabolomics, and transcriptomics, we propose to elucidate the unexplored role of ECM biochemical properties in vascular cells reprogramming during PAH. Our preliminary data identify Nidogen-2 (NID2) as a key ECM protein upregulated in multiple PAH models and human subjects. Also, our results indicate that modulating its expression reprograms vascular cells proliferation. Therefore, we hypothesize that NID2 perivascular upregulation reprograms pulmonary vascular cells behavior to promote PAH. By unveiling its crucial role in connecting vascular wall structure to vascular cells behaviors, we will provide critical insights into the molecular underpinning of PAH with new therapeutic perspectives.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-13

Cancer / System bio **CHALABI Asma** IPMC

Dynamic analysis framework to detect cell division and cell death in live-cell imaging, using signal processing and machine learning

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Cell division and cell death are the main indicators for cancer drug action, and only their accurate measures can reveal the actual potency and efficacy of a compound. The detection of cell division[1] and cell death[2,3] events in live-cell assays has the potential to produce robust metrics of drug pharmacodynamics and return a more comprehensive understanding of tumor cells responses to cancer therapeutic combinations. Yet, classical methods require dyes to measure cell viability as an end-point assay, in which proliferation rates and cell division events are often not measured.

Live-cell imaging is a promising cell-based assay to determine drug efficacies, with the main limitation being the actual accuracy and depth of the analyses to acquire automatic measures of the cellular response phenotypes, cell death and division, which share some morphological features. In this work, we present a new algorithmic architecture integrating machine learning, image and signal processing methods to perform dynamic image analyses of single cell events in time-lapse microscopy experiments of drug pharmacological profiling. Our event detection method is based on a pattern detection approach on the bright field signal entropy making it free of any labeling step and exhibiting two distinct patterns for cell division and death events. Our analysis framework is an open source and adaptable workflow that automatically predicts cellular events (and their times) from each single cell trajectory, along with other classic cellular features of cell image analyses, as a promising solution in pharmacodynamics.

The framework is validated on HeLa cells with an accuracy > 95% of good phenotyping capacity. The ongoing work aims to extend the use of the algorithm and to optimize it for other cancer cell lines of a Human Cancer Cell line Panel (HCCP) to improve our live-cell OMICS profiling approaches[4], and to scale up pharmacological screening of new cancer drugs.

Keywords: single cell, live-cell, phenotype, machine learning, image and signal processing, pharmacodynamics.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-14

Immuno **BENHMAMMOUCH-CARMINO Saloua** C3M

Glutamine synthesis and production pathways control metabolic and functional plasticity of macrophages.

Glutamine metabolism is considered as a “fuel for the immune system” and *in vitro* studies have demonstrated its repair role in macrophages (Jha et al., 2015). Our team has demonstrated that glutaminase Gls1, hydrolyzing glutamine into glutamate, is a limiting step in this process *in vitro* and *in vivo* by promoting clearance of apoptotic process called efferocytosis (Merlin et al., 2021). Glutamine depletion in macrophage culture medium leads to Gls1 deficiency independent effects *in vitro*. Besides Gls1, macrophages express another isoform called Gls2 and can synthesize glutamine through glutamine synthase (Gs). We hypothesized that these enzymes could contribute to the metabolic and functional plasticity of macrophages. To test this hypothesis, we generated Gls1 and/or Gls2 deficient macrophages and modulated GS activity by the inhibitor methionine sulfoximine (MSO) in these cells. *In vitro*, metabolic activity was measured by Seahorse analysis whereas their functionality was tested after interleukin 4 stimulation by flow cytometry and efferocytosis measurement. After IL-4 stimulation, we confirmed that the invalidation of Gls1 dependant glutaminolysis in macrophages leads to a decrease of oxidative phosphorylation as well as repair response markers (CD206, PDL-2) and efferocytosis. These effects were not observed in Gls2 deficient macrophages, whether in the presence or absence of Gls1. It suggests a minor role of Gls2 in macrophage repair response. Unexpectedly, the inhibition of Gs with MSO, reduced the metabolism and macrophages repair response both in control cells and in Gls1 deficient cells, suggesting a complementarity between synthesis and production pathways. RNA sequencing helped us to demonstrate that GLS1 inhibition led to a completely different transcriptional reprogramming from GS inhibition. Our preliminary results suggest that if the invalidation of Gls1 leads to a decrease in cellular glutamate, the inhibition of GS would rather reduce the levels of cellular glutamine, a precursor of the hexosamines pathway. Our results suggest that the macrophage modulates its cellular glutamine fluxes to adapt its metabolic and functional plasticity under reparative conditions. We are currently testing the relevant role of these enzymes *in vivo* through chronic inflammatory models of diabetes and atherosclerosis.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-15

Developmental bio / Neuro **FJERDINGSTAD Niels** iBV

Potential link between Interpeduncular Nucleus plasticity and susceptibility to anxiety

Facing trauma, some adults will develop an anxiety disorder, but others will not. Why are some individuals more vulnerable to psychiatric illnesses?

The Neurodevelopmental hypothesis suggests that at the roots of these disorders lies a developmental flaw in the involved neuronal networks, the Reward System, at a precise timeframe named critical period. During these periods, which often take place during childhood or adolescence, neuronal networks undergo a highly plastic that is essential to their maturation as they will shape themselves under the influence of environmental factors. During these periods, abnormal stimuli such as traumas, can alter the maturation of the involved neuronal networks and lead to long-term susceptibility, which would later be revealed during a new trauma.

Aiming to model this phenomenon, we have found that a moderate chronic stress inflicted at a specific timeframe, during puberty, leads to susceptibility to chronic anxiety, by disrupting the Habenulo-Interpeduncular-System (HIPS), a central element of the Reward System. A second round of stress in the adult then triggers the pathology onset with the apparition of anxiety-like behavioural symptoms. Therefore, our model replicates perfectly what is commonly observed in humans.

To examine the underlying mechanisms, perineuronal nets (PNN) are now the focal point of my thesis project, as they play a major role in both the onset and closure of critical periods, and are key actors of the regulation of brain plasticity. PNN are a condensed form of extracellular matrix that enwrap a subset of neurons during critical periods. I have established their presence within the HIPS, and now aim to see if they play a role in the HIPS-dependent susceptibility to anxiety. My hypothesis is that their maturation is altered during a moderate chronic stress, which can impact the long-term functioning of the HIPS. Therefore, I will analyse whether a juvenile or adult stress has an impact on the state of PNN in the HIPS. I will also investigate whether their degradation modifies the HIPS response to stress. These experiments will help determine whether the PNN in the HIPS play a role in the HIPS-dependent susceptibility to anxiety, as well as establish if their manipulation can help protect from this susceptibility.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-16

Cancer | **GUO Yingbo** | C3M

Primary cilium and prostate cancer

Stabilization of Hypoxia-Inducible Factor-1α (HIF-1α) in hypoxia results in truncation of the mitochondrial voltage-dependent anion channel C-terminal (VDAC1) into VDAC1-ΔC. Recently, our team linked the regulation of ciliogenesis, processes by which the primary cilium (PC) is formed, to VDAC1-ΔC and tumor aggressiveness.

In clear cell renal cell carcinoma (ccRCC), kidney cancer, we characterized a PC signature with two markers, GLI1/IFT20. The presence of PC and the two markers Gli1+/IFT20+ was associated with more aggressive cancer and patients died faster. Our hypothesis is that such a signature can be extended to other types of cancer, including prostate cancer (PCa), which is described as no primary cilia expression. 98% of PCa patients have adenocarcinoma prostate cancer (AdPC) firstly and 20% of AdPC are induced into neuroendocrine prostate cancer (NEPC) after long-term treatment, which is the worst PCa subtype. By using TCGA cancer genomics program, we characterized, in a cohort of 550 AdPC patients, expressing the GLI1+/IFT20+ signature. Do the PCa cells of these patients express PC and are the PCa of these patients more aggressive? Our objectives are therefore to mimic this condition to detect the link among PC, GLI1/IFT20 and PCa aggressiveness.

Normal cell line P69, prostate adenocarcinoma cell lines 22Rv1, LNCaP, DU145, PC3 and neuroendocrine cell line NCI-H660 were used as 2D cell models. 3D models include (1) RWPE1 normal cells forming acini, containing a lumen, mimicking prostate gland and (2) WPE1-NB26 tumoral cells forming a tumoroid-like structure. The presence of PC was detected by colocalization of Arl13B and acetylated □-tubulin via immunofluorescence. Gefitinib, an inhibitor of EGFR was used to restore PC as described by Khan et al. (2016). Different O2 concentrations were used to culture cells: normoxia (Nx) 21%- and hypoxia (Hx) 1%- O2.

In 2D culture, in Nx, a low percentage of PCs were detected in P69 (9%), RWPE1 (4%) and WPE-NB26 (1%) cells, none in adenocarcinoma cells but a high percentage (60%) in neuroendocrine cells. In Hx, the percentage of ciliated cells was reduced correlated with the presence of VDAC1-ΔC. Normal cells did not express PC anymore and NCI-H660 cells only expressed 10% of PC. Gefitinib increased the percentage of PC in P69 into 14% significantly, whereas RWPE and WPE1-NB26 expressed 60% of PC. Like ccRCC, cells expressing PC exhibited a Gli1+/IFT20+ signature.

In 3D, PC was detected in both acini and tumoroid formations. In the presence of gefitinib, the 3D growth of both models was inhibited, and the lumen of acini disappeared. The impact of the PC in this treatment is currently being evaluated.

Our study suggests that the re-expression of PC, associated with the GLI1+/IFT20+ signature, can be used as a diagnostic basis to detect the NED early and propose a better personal therapy.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-17

Bioinformatics / Omics | **BILLON Victor** | IRCAN

How genes endure intronic transposable elements.

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LINE-1 (L1) retrotransposons are mobile genes that can copy-paste themselves at new locations in their host genome. During evolution, they spread in the human genome and now compose a great part of human introns. L1s contain transcriptional characteristics similar to the gene they are embedded in. Consequently, new insertions can cause rare genetic diseases. However, human introns contain hundreds of thousands of intronic L1s not known to cause transcriptional interferences raising the question of L1 impact on gene transcription. In my work I am focusing on the role of L1 on transcriptional attenuation, which is the detachment of the RNA polymerase II from the gene template.

Two hypotheses could explain why only some L1s can attenuate transcription: (i) intronic L1 sequences could be globally detrimental to gene expression but their effect masked by suppressors of attenuation ; (ii) L1s could be neutral to genes but the presence of triggers would lead to locus and cell-type specific attenuation.

To explore these two hypotheses and study the extent of L1 effect on genes, I developed a bioinformatic screen to identify intronic L1s associated with attenuated genes. This screen, based on nascent transcript sequencing technologies, showed that the presence of an intronic L1 is not often correlated with gene attenuation. Then, looking at L1 integrity in introns, I showed that sense oriented L1s are depleted of their first 2000 nucleotides and enriched of their last 500 nucleotides, suggesting that specific segments of L1 could be detrimental.

Finally, I designed a reporter system to study attenuation in cell culture and I successfully integrated it in a gene potentially attenuated by a L1. This system can be used to study molecular mechanism linked to attenuation.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-18

Cancer / Immuno **KRUG Adrien** C3M

Metabolic addiction to mitochondrial respiration of malignant PD1high Tfh cells reveals a new therapeutic target for Angioimmunoblastic T Cell Lymphoma

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Cancer metabolic reprogramming has been recognized as one of the cancer hallmarks that promote cell proliferation, survival, as well as therapeutic resistance. However, the regulation of metabolism in T cell lymphoma is poorly understood. We demonstrated here that PD-1 co-inhibitory signals co-incided with a change in metabolic phenotype in angioimmunoblastic T cell lymphoma (AITL). Through genomic analysis of clinical human AITL data, we identified that AITL tumor cells use OXPHOS metabolism and reactions to fulfill their energetic requirement. By using our pre-clinical mouse model plck-GAPDH mimicking closely human AITL features, we confirmed that PD-1^{high} Tfh tumor cells exhibit a reduction of glycolytic flux *in vivo* associated with a strong enrichment of OXPHOS metabolic signatures, high mitochondrial content and ROS accumulation. Consistent with these results, disruption of OXPHOS metabolism using an inhibitor of mitochondrial electron transport chain complex like metformin or a ROS scavenger improved survival of AITL lymphoma-bearing mice. *In vitro* we confirmed a selective elimination of the PD-1^{high} Tfh human AITL cells upon ETC inhibition. In agreement, diabetic patients suffering from T cell lymphoma, treated with metformin survived longer as compared to patients receiving alternative treatment. Taking together, our finding suggests that targeting this OXPHOS pathway might be a clinically efficient approach to inhibit lymphoma progression in AITL disease.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-19

Physio - Electrophy **DUNOT Jade** IPMC

Aeta peptide levels dictate long-term depression and impact spatial memory

Over the past thirty years, studies on the amyloid precursor protein (APP) have primarily focused on the synaptotoxicity of one of its fragments, the amyloid-beta peptide (Aβ), in Alzheimer's disease. However, APP, physiologically cleaved, allows the secretion of Aß but also of other peptides, including the peptides secreted from the η-secretase pathway (Aη and sAPPη) described by Dr. Marie with his German collaborators recently (Willem et al., Nature 2015). Since this publication the team has obtained solid preliminary data allowing to formulate the hypothesis that these peptides alter synaptic communication and that the APP η-secretase pathway is necessary for ancient spatial memory. This project proposes to elucidate a major unresolved question: what is the physiopathological role of these peptides secreted from the APP η-secretase pathway in the hippocampus? In order to answer this question, two new mouse model were created. The first one, named MISEPA2, correspond to a chronical increased of the human Aη-α levels in the brain. The second one, named APPΔη, correspond to a deletion of the η-secretase pathway using CRISPR/Cas9 method. Behavioural tests were carried out on these two models after a western blot characterisation of the APP peptides. We also use ex-vivo electrophysiology to analyse the synaptic plasticity. MISEPA2 mice have no modification in the levels of the APP peptides even Aβ is normal. With the Morris Water maze test, mice have a slightly alteration of the spatial memory one week after the last training day when 24 hours after the spatial memory is normal. The long-term potentiation and long-term depression are also altered in these mice in function of the levels of magnesium and calcium in the aCSF. APPΔη mice present some modifications in the levels of sAPP in the brain but Aβ is normal. In these mice, the long-term potentiation is normal but the long-term depression is altered in male and there is also some memory alterations. It possible to rescue the long-term depression by incubating hippocampal slices with the synthetic Aη-α peptide. These results show that the acute or chronic increase in Aη-α modifies excitatory synaptic plasticity and the associated memorization processed. We also validate the hypothesis that the peptides secreted from the η-secretase pathway exhibit an important physiological function for the endogenous modulation of synaptic communication.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-20

Cancer | **PISIBON Céline** | C3M

Role of epigenetic alterations in cutaneous melanoma resistances to targeted therapies

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Cutaneous melanoma (15,500 new cases per year in France) is a cancer that derives from melanocytes, cells responsible for skin pigmentation. If diagnosed early, its prognosis is favourable but at the metastatic stage it remains very poor. Despite progress of metastatic cutaneous melanoma treatments with targeted therapies and immunotherapies, more than half of patients experiences only short-term or no benefit of these therapies due to innate or acquired resistances. Our team has shown that melanoma cells are able to adapt and evade targeted therapies. It is therefore necessary to find new therapeutic approaches to prevent or overcome these resistances whose causes are often non-genetic and rely on the phenotypic plasticity of melanoma cells. Indeed, resistance to targeted therapies is associated with epigenetic reprogramming leading to the loss of MITF (Microphtalmia-associated transcription factor, master gene of melanocyte development), dedifferentiation and an increase in the mesenchymal phenotype. These site-specific epigenetic alterations may be explained by the regulation of chromatin remodeling complexes. Our candidate protein may be involved in these processes as it interacts with chromatin remodelers, including histone deacetylases and histone methyltransferases, and key epithelial-mesenchymal transition (EMT) transcription factors. Using loss of function and pharmacological approaches, we investigated the impact of our protein inhibition on the responsiveness of targeted therapies-resistant melanoma cells to therapies targeting the MAP kinase pathway. Using shRNA, we demonstrated that the knockdown of our target increases MITF expression, decreases mesenchymal markers expression, impairs migration of resistant melanoma cells and resensitizes cells to BRAF inhibitor. Moreover, subcutaneous xenograft of resistant melanoma showed a better survival of mice that have received knockdown cells for our protein of interest and targeted therapies treatment. In agreement with these observations, pharmacological inhibition of our protein reduces proliferation therefore seems to resensitize resistant cells to BRAF inhibitor. Given these results, our protein, which plays a key role in melanoma cell plasticity and resistance to targeted therapies, could be a rational therapeutic target to overcome resistance to targeted therapies and improve melanoma treatments.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-21

Cancer | **NICOLINI Victoria** | IRCAN

Glucocorticoid receptor negatively regulates Processing-body formation

Processing-bodies (Pbodies) are described as small cytoplasmic membraneless organelles that play an important role in various cellular processes by controlling RNA translation. Pbodies are formed by the interaction of untranslated mRNA and multiple RNA binding proteins assembled by liquid-liquid phase separation (LLPS). However, despite recent discoveries about their own key components, the cellular pathways that control their formation or dissolution are poorly understood.

In this context, we have conducted an extensive drug screening to identify targets able to enhance Pbody formation. In collaboration with the PCBIS facility, we uncover that glucocorticoids were able to increase the number of P-bodies in cells after 48 hours. By combining microscopy and biochemistry experiments, we surprisingly found that this increased number of PBodies was associated with the decreased expression of the glucocorticoid receptor (GR) in response to its ligand in a dose-dependent manner. Consistent with this finding, we also showed that siGR led to an increase in the number of PBodies, demonstrating that the formation of PBodies is independent of GR induced transcription. Based on these results, we hypothesized that the cytoplasmic location of GR has a negative effect on the number of Pbodies. Namely, when GR is activated, it is translocated to the nucleus, and when we use siRNA against GR, there is less GR in the cytoplasm, where the Pbodies are located. It is now important to validate this hypothesis to better understand the relationship between the decrease in protein content of GR and the increase in the number of Pbodies in the cells. Overall, our results illustrate a non-canonical function of GR, which regulates RNA translation through a cytoplasmic LLPS.

Keywords: Processing-bodies (Pbodies), Glucocorticoid Receptor (GR), Glucocorticoid, Liquid-Liquid Phase Separation (LLPS)

ORAL COMMUNICATIONS

3rd year PhD Students

OC-22

Cancer / Dev Bio | **FARNET Aurélie** | iBV

OTX2, a potential pharmacological target for medulloblastoma

OTX2 (orthodenticle homeobox 2) is a transcription factor involved in many developmental processes such as gastrulation, development of the cerebellum and several sensory organs. OTX2 is also involved in the development of medulloblastoma (MB), a pediatric cancer of the cerebellum that represents about 20% of pediatric tumors in the central nervous system. That cancer is subdivided into 4 subgroups according to the molecular characteristics of the tumor cells : WNT activated, SHH activated, group 3 and group 4. Abnormal over-expressions and genomic amplifications of OTX2 have been found in group 3 medulloblastoma (MB3) which is poorly characterized and particularly agressive with a 50% survival rate at 5 years. Today, the therapeutic resources are based on surgery accompanied by heavy treatments such as radiotherapy and chemotherapy which can leave profound neurological and neurocognitive sequelae. It is therefore necessary to develop new approaches to improve the medical care of this devastating disease. In our laboratory, we have demonstrated that OTX2 is involved in cerebellar tumorigenesis. Indeed, functional experiments on a MB3 cell line have shown that OTX2 over-expression induced an increase in cell proliferation and polyploidy. On the contrary, a decrease of OTX2 through the use of anti-OTX2 siRNAs and shRNAs induces a considerable reduction in proliferation and polyploidy. These observations suggest that a decrease of OTX2 in MB3 would help control the tumoral growth, and therefore constitutes an interesting therapeutic target. However, the therapeutic use of anti-OTX2 siRNAs and shRNAs remains limited due to their high instability and difficulty in reaching the target cells. An alternative would be to use pharmacological compounds capable of inhibiting OTX2 expression in these tumors, which can be administered orally or parenterally. Unfortunately, no chemical inhibitors of OTX2 have been described so far. In order to identify such molecules, we have carried out an analysis of 1200 pharmacological molecules by RTqPCR on MB3 cells, which has allowed us to obtain several interesting molecules that have an inhibitory impact on the expression of OTX2. I now wish to analyze in greater depth their mechanism of action.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-23

28 *Biochemistry* **SAFWAN ZAITER Hasan** iBV

The senescence marker P16Ink4a a player of liver endothelial cells physiology

P16INK4A is a tumor suppressor and cell cycle regulator that has been linked to aging and senescence. In development, a potential role of p21 and of p19ARF has been postulated, but little is known about p16. Our previous results revealed a highly dynamic expression pattern of p16 in development and in different organs and cell types assessed by qRTPCR and immunohistochemistry (IHC). In addition, we also noticed through IHC observation that p16 expression in old liver is mainly in the endothelial cells (ECs) compared to parenchymal cells. Therefore, we aimed at better understanding the role of p16 in biological processes of liver ECs, such as proliferation, migration, apoptosis, and tube formation. We also performed RNA sequencing to identify genes differentially expressed between young and old ECs.We used small hairpin (shRNA) constructs and a p16 cDNA-GFP vector to knockdown and overexpress p16 in vitro,in two types of liver ECs, CD31+ vascular ECs and CD146+ sinusoidal endothelial cells. Afterwards, we assessed p16'sdown- and up-regulation effect on ECs function. Brdu incorporation assays showed that p16 upregulation wasassociated with slower proliferation compared to control cells, whereas its downregulation induced higherproliferation compared to control cells. Scratch assay and transwell migration assays showed attenuated migration in p16 overexpressed cells compared to baseline expression, while only transwell assays showed the ameliorated migration of p16 knockdown cells compared to controls. Similar migration between p16 knockdown and control was observed in scratch assays. We also observed in β-gal staining, a marker of senescence, a higher number of stained cells in p16 overexpression conditions compared to controls, while less cells were stained in the case of knockdown. Additional experiments that aim to further decipher p16's effect in ECs' tube formation, apoptosis, and telomeres shortening are ongoing, which might contribute to the invention of more specialized anti-aging therapies.

Keywords: aging, development, endothelial cells, liver, p16, senescence,

ORAL COMMUNICATIONS

3rd year PhD Students

OC-24

Bio modeling | **GUICHARNAUD Chloé** | ISA

Pushed to the edge: ecological and evolutionary dynamics of pushed vs. pulled expansions in micro-wasps

While species ranges have always moved, the ecological and evolutionary dynamics of range expansions have become especially relevant today, as human influence reshapes communities worldwide. Contrary to classical “pulled” dynamics, in which the low-density front populations of an expansion are the main source pulling the rest of the expanding population to expand, some expansions exhibit dynamics in which high-density rear populations, behind the front, actually “push” the expansion forward. Combining the pushed/pulled expansion concept with evolution occurring at range edges highlights the importance of understanding how density-dependent dispersal or growth evolves during expansions. It has been suggested that not accounting for this may explain failures to predict colonization dynamics of e.g. invasive species or biocontrol agents. However, the comparative ecological and evolutionary consequences of pushed versus pulled dynamics remain to be studied, especially in the context of pre-existing life-history differences. To better understand these dynamics, we did create replicated range expansions using colonization of experimental landscapes by Trichogramma micro-wasps, a biocontrol agent. We will compare the ecological and evolutionary dynamics of strains and species varying in dispersal abilities, dispersal density-dependence and association of these traits with life-history traits. This will allow us to determine to what extent the evolution of density-dependent dispersal is predictable from initial trait architecture, and the consequences for the fate of pushed vs pulled expansions.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-25

Cancer | **TEISSEIRE Manon** | IRCAN

CTGF : A new therapeutic strategy in metastatic renal cell carcinoma ?

Manon TEISSEIRE[1], Julien PAROLA[1], Maëva TOTOBESOLA[1], Frédéric LUCIANO[1], Gilles PAGÈS[1] and Sandy GIULIANO[1]

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Among the various tumors, kidney cancer and in particular clear cell renal cell carcinoma (RCC), the most common form of kidney cancer, is probably one of the most vascularized tumors. The recurrent inactivation of the von Hippel Lindau gene which leads to the stabilization of Hypoxia Inducible Factor 1 and 2 a (HIF1/2a), is a major cause of this hypervascularization. VHL inactivation is key for the expression of one of the most important pro-angiogenic factors, vascular endothelial growth factor (VEGF). Sunitinib and axitinib are two tyrosine kinase inhibitors used in metastatic renal cell carcinoma (mRCC). They prolong progression-free-survival (PFS) in patients with mRCC. Unfortunately, in most of the cases, patients relapse after one year of treatment. The anti-angiogenic role of sunitinib on endothelial cell is well described, but its role on tumor cells is poorly understood. Previous results described sunitinib as a lysosomotropic agent which disrupts autophagy pathway (Giuliano et al., Autophagy, 2015). In the literature, autophagy inhibition creates a pro-inflammatory environment. Proteomic analysis of sunitinib treated and resistant cells to this treatment showed an increase in pro-inflammatory proteins and secreted factors (Giuliano et al., Theranostics, 2019). Among them, we identified Connective Tissue Growth Factor (CTGF). Interestingly, RNA sequencing and proteomic analyses of axitinib treated and resistant cells also showed an increase in CTGF. CTGF is a signaling factor which promotes cancer initiation, progression and metastasis by regulating cell proliferation, migration and drug resistance. We hypothesize that CTGF is implicated in anti-angiogenic resistance.

We first demonstrated an increase of i) CTGF mRNA levels (RT-qPCR), and ii) CTGF secreted form (ELISA assay) in mRCC sunitinib treated and resistant cells, and similar results were obtained with axitinib treated and resistant cells. We also demonstrated that CTGF invalidation by siRNA decreases proliferation, migration and invasion of non-treated, sunitinib, axitinib treated cells and in sunitinib, axitinib resistant mRCC cells. Moreover, CTGF invalidation induces cell apoptosis reversed by Q-VD-OPh, a caspase specific inhibitor. Finally, CTGF recombinant protein increases mRCC cell migration. Our results indicate that CTGF may play a key role in mRCC sunitinib and axitinib resistance but also in tumor cell aggressiveness and need to be further investigate.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-26

Immuno | **DOLFI Bastien** | LP2M

Phenotypic and functional analysis of sexual dimorphism in adrenal gland macrophages

Macrophages are innate immune cells found in all organs. Macrophages are the first line of host defense against pathogens. Recently, it was reported that macrophages display great phenotypic and functional heterogeneity. Indeed, the progress of numerous technologies of analysis during the last decades, coupled with the improvement of flow cytometry and microscopy, highlighted heterogeneity in tissue macrophage populations. Thus, the presence of various macrophage subsets characterized by the expression of specific markers was revealed in tissues. This diversity could be explained, at least partially, by their developmental origin. Moreover, the local microenvironment also plays a major role in shaping macrophage diversity. Furthermore, biological sex has been shown to influence myeloid cell diversity. This could account for the increased prevalence of several autoimmune diseases in women.

My work was focused on characterizing immune cell diversity in the adrenal glands. We observed that adrenal glands contained multiple macrophage subsets with various developmental origins. Moreover, we highlighted a sexual dimorphism in their location and phenotype. Macrophage depletion had a strong impact on adrenal gland hormones, which underlies their critical role in the maintenance of tissue homeostasis. Finally, we aimed to define how stress could influence the immune cell response and found a difference in immune responses to “acute” and “chronic” stress between males and females.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-27

Neurobiology **CANAGUIER Juliette** IPMC

The microbial metabolite p-cresol induces social interaction deficits in mice by a potential deregulation of catecholamines biosynthesis

Juliette Canaguier [1, 2] , Jean-Marie Launay[3,4] , Jacques Callebert[3,4] , Nicolas Glaichenhaus[1, 2] and Laetitia Davidovic[1, 2]

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Autism spectrum disorders (ASD) are frequent neurodevelopmental pathologies characterized by social behaviour and communication deficits, repetitive behaviours, and restricted interests. Some ASD patients also display gastro-intestinal symptoms, intestinal dysbiosis and abnormal levels of microbial metabolites. Studies in rodents have shown that both bacteria from the microbiota and microbial metabolites regulate brain function and behaviour, including social behaviour. These findings suggest that disruption of the microbiota-gut-brain axis could contribute to the development and/or maintenance of ASD symptoms. In the recent years, we have focused on the microbial metabolite *p*-cresol that is described as abnormally elevated in the feces and urine of ASD patients (1, 2). We have previously shown that C57Bl/6 mice exposed chronically to *p*-cresol exhibit severe social interaction deficits, perseverative behaviours, and stereotypies, which are reminiscent of core ASD symptoms (3). This is accompanied by a decrease in midbrain dopamine (DA) neurons excitability (3), suggesting that p-cresol effects on behaviour may relate to perturbations of catecholamines within the social reward circuit. Previous *in vitro* studies have shown that p-Cresol inhibits dopamine beta-hydroxylase (DBH), a key enzyme in the synthesis of norepinephrine (NE) which converts DA to NE (4). We therefore hypothesized that p-cresol-induced social behaviour deficits are due to a direct effect of p-cresol on brain catecholamines biosynthesis.

To test this hypothesis, we measured the central levels of p-Cresol, DA, NE, and the activity of key enzymes for DA and NE synthesis: tyrosine hydroxylase (TH) and DBH. We observe an increase in p-Cresol, a decrease in DA and NE, an increase in DA turnover, and a decrease in TH and DBH activity in the brainstem of p-Cresol-treated mice. Also, the levels of DA and NE as well as DBH activity correlate with the severity of social interaction deficits. Finally, acute pharmacological inhibition of DBH results in social interaction deficits in C57Bl/6 mice. This suggests that deregulation of catecholamine biosynthesis, and in particular of NE, may be responsible for the deleterious effects of p-cresol on the reward circuit and social behaviour. Our data support the notion that some microbial metabolites can modulate social behaviour by a direct modulation of neurotransmitter synthesis.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-28

Cancer / Immuno **ELALDI Roxane** IPMC

Spatial Characterization of invasive cutaneous squamous cell carcinoma by multiparametric imaging mass cytometry

Roxane Elaldi, Aïda Meghraoui-Kheddar, Axel Elaldi, Alizé Gouleau, Patrice Hemon, Luciana Petti, Anne Sudaka, Gilles Poissonnet, Jacques-Olivier Pers, Véronique Braud, Fabienne Anjuère

Introduction: Cutaneous squamous cell carcinomas (cSCC) are the 2nd deadliest skin cancer. They are currently treated by excisional surgery but can reach, for some patients, a non-operable stage associated with rapid local and nodal relapses and a very poor prognosis. However, no consensus has been reached on the clinical or molecular factors predicting these recurrences. It is therefore crucial to improve the identification of patients that would relapse by the characterization of prognostic biomarkers. An integrative spatial characterization of cSCC immune microenvironment and the interactions of their components are required to identify such biomarkers.

Objective: The aim of our study is to obtain an exhaustive and integrative characterization of the immune microenvironment (TiME) of recurrent and non-recurrent cSCC, including the interaction maps between the main actors described to be associated to tumor progression. The comparison of the specific signature of these two cSCC groups will allow the identification of prognostic biomarker candidates that could help predict relapses.

Methodology: A cohort of 25 cSCCs with different prognosis was constituted including one group of non-relapsing tumors and 2 groups of initial tumors having a local relapse or a nodal relapse at 2 years. For every tumors except one, perilesional skin was also analyzed. An imaging mass cytometry (IMC) panel of 39 antibodies targeting components of the cSCC TiME (tumor cells, immune subtypes, blood and lymphatic vessels, extracellular matrix and nerves fibers) was used to stain a section of each formalin fixed and paraffin-embedded (FFPE) tumor of the cohort. Each section was analyzed by IMC and the 40-dimensional images obtained were processed using an in-house developed analysis pipeline.

Results and discussion: IMC image preprocessing and computational analysis of the single cell phenotypes extracted from the images allowed *(1)* the identification of each targeted TiME component subset, from tumor cells to both myeloid and lymphoid immune cells, *(2)* in addition to their functional status (proliferation, apoptosis, exhaustion) and *(3)* their localization within tumor structures. This analysis led to the identification of specific cell composition and spatial features characterizing the TiME of cSCCs, compared to perilesional skin. The comparison of the specific signature corresponding to each tumor group with different prognosis uncovered a predictive biomarker associated with relapse risk after surgical excision of cSCC tumors that will be confirmed in an independent validation cohor

ORAL COMMUNICATIONS

3rd year PhD Students

OC-29

Developmental bio / Physio **HERAULT Chloé** iBV

Functional characterization of new sex pathways downstream of the female sex determinant TransformerF

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An individual can be characterized by the presence of particular sex organs: testes or ovaries. The formation of these reproductive organs is controlled by specific genetic elements: the sex chromosomes. However, sex differences encompass much more than sex organs. Evidence suggests that males and females differ in their normal physiology and susceptibility to various diseases. *Drosophila* is one of the best-characterized systems for the molecular mechanisms that govern sex determination. The females are XX and the males are XY. The detection of sex chromosomes activates a splicing cascade that results in the production of the sex determinant TransformerF (TraF) only in females. This female sex determinant controls the sexual identity of the gonads and is never expressed in males. Until now, researchers considered adult flies of both sexes to be mosaics of cells knowing their sexual identity (like the cells of the gonads) and cells not knowing their sexual identity (the majority of cells). Using fly models, my team demonstrated that the sexual identity of intestinal stem cells plays a key role in the adult gut for the sex-specific pre-disposition to tumours, highlighting the importance of a new cell-intrinsic mechanism. While these findings establish the proof-of-principle of the influence of sex chromosomes in adult cells, essential gaps remain to be filled. Indeed, the full range of phenotypic consequences of the presence of sex chromosomes in somatic cells, the genes, the mechanisms involved, and their sites of action remain entirely elusive. My project aims to understand where and how the intrinsic presence of sex chromosomes, the cellular sex, impacts physiology across the body using Drosophila melanogaster as an in vivo model. Surprisingly, I discovered that all the organs, from embryonic to adult stages, have an intrinsic sexual identity, which can be visualized by the expression of the female sex determinant TraF. I showed that TraF is necessary and sufficient to drive overall sex differences in phenotypes like body size and weight. By focusing on the sex differences in size, I showed that females are larger than males because their organs have more cells. Indeed, autonomously, the cellular sex shortens the cell cycle duration in females during a critical developmental period.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-30

Neurobiology **PLONKA Alexandra** CoBTeK

Early and differential diagnosis of Primary Progressive Aphasia: Contribution of graphic and gestural markers.

Key words : Primary Progressive Aphasia, Diagnosis, Graphic markers, Gestural markers

Primary Progressive Aphasia (PPA) is a neurodegenerative syndrome initially characterized by an isolated language disorder and which diagnosis is mainly clinical. Three main PPA subtypes have been described in the literature: logopenic PPA, semantic PPA, and non-fluent/agrammatic PPA (nfavPPA) [1]. The latest data in the literature have highlighted a late diagnosis, sometimes erroneous, associated with the need for an early medical-care management [2]. The creation of new tools for the diagnosis and classification of PPAs seems to be crucial for an early and adapted patients' care.

The objective of this work is to verify the interest of the analysis of markers using new technologies such as motor activity parameters (graphic and gestural) within the speech therapy evaluation, to improve the early and differential diagnosis of PPAs.

Initially, this work made it possible to analyze the specificities of the PPA diagnosis among the 167,191 diagnoses recorded in the French National Alzheimer Bank between 2010 and 2016. We highlighted diagnostic wavering and misdiagnosis of patients with PPA [3]. These results underline the importance of developing tools that can be integrated into speech therapy practice and that can allow a greater accuracy in diagnosis.

Following these observations, we have demonstrated the interest of using new technologies that can be easily integrated into a diagnostic approach. By using touch tablets, we have demonstrated the interest of the analysis of graphic parameters, such as writing pressure and strokes, for the differential diagnosis of PPA patients in comparison with patients with Alzheimer's Disease (AD) or patient with Posterior Cortical Atrophy [4], [5]. These parameters have also proven to be useful in the classification of the three main subtypes of PPA [6]. To complete the evaluation of strokes for which the gestural behaviors during the pencil lifting times cannot be directly recovered on an electronic tablet, we were interested in the evaluation of the writing gesture by video analysis. Our analysis showed a complementary interest for the more specific diagnosis of the PPA non-fluent variant.

The objective is now to validate these results on a larger cohort of patients to create an automated tool for processing handwriting parameters and ease PPA diagnosis.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-31

Biochemistry **AL-QATABI Noha** IPMC

Functional characterization of TgREMIND, an enigmatic F-BAR-containing protein involved in vesicular trafficking in Toxoplasma gondii

Noha Al-Qatabi[1], Sophie Pagnotta, Maud Magdeleine[2], Sandra Lacas-Gervais[2] and Guillaume Drin[1]

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Toxoplasma gondii, the causative agent of toxoplasmosis, is an obligate intracellular parasite. The ability of this parasite to infect host cells depends on its capacity to release unique factors from special organelles called rhoptries, micronemes, and dense granules. The biogenesis of these organelles relies on vesicular trafficking events whose molecular bases are poorly known. In eukaryotic cells, key players of vesicular trafficking are BAR-containing proteins with a capacity to bind and sense/induce membrane curvature, and to recruit protein partners; however, whether BAR-containing proteins play pivotal roles in *T. gondii* is unknown. Here we characterize a BAR-containing protein called *Tg*REMIND involved in the delivery of rhoptries and dense granule proteins into their destination compartments, and its absence lead to the inability of the parasite to be infectious. Bioinformatics analyses suggest that *Tg*REMIND has a putative F-BAR domain and a domain, referred to as X-REMIND, whose fold and function are unknown. Using circular dichroism and flotation assays, we determined that *Tg*REMIND contains a functional F-BAR domain that binds membranes that are both curved and enriched with phosphoinositide (e.g., $PI(4,5)P_2$), possibly via particular basic residues. Moreover, we found by electron microscopy that this F-BAR domain can induce membrane tubulation. In parallel, we found that the X-REMIND domain is well-folded, and obtained experimental data supporting AlphaFold predictions. We found that this domain neither binds nor remodels membrane yet obtained preliminary data suggesting that it might regulate the membrane remodeling capacity of the full-length *Tg*REMIND. Overall our data provide first clues on the function of a BAR-containing protein from the Apicomplexan phylum.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-32

Cancer | **SERVANT Zoé** | iBV

Fas activation leads to late endosomal Fas trafficking and exosomes formation

The development of cancer is favored by an imbalance between cell renewal and cell death, with proliferation being favored whereas cell death being inhibited. However, several studies show that cell apoptosis has the capacity to endow dying cells the ability to promote proliferation of neighboring cells. These data suggest that tumor cell apoptosis may influence tumor environment by secreting various signals including soluble factors, cell debris and extracellular vesicles (EV), which regulate tumor growth. Indeed, secretion of EV plays a key role in tumorigenesis by modulating the immune response against the tumor or by promoting tumor growth and metastatic dissemination.

Our recent data demonstrate that the death receptor Fas, a potent inducer of cell death by apoptosis, interacts directly with Syntenin-1, a component involved in the biogenesis of a type of EV called Exosomes, suggesting that Fas activation by its ligand (FasL) and the following induction of apoptosis may favor secretion of this type of EV. Exosomes are intraluminal vesicles (ILV) formed by inward budding of the endosomal membrane during maturation of the multivesicular endosomal compartment (MVB) and secreted after fusion of the MVB with the plasma membrane of the cell.

We show that Fas activation by FasL generates a strong secretion of Exosomes which express Fas at their surface. The biogenesis of Fas-dependent Exosomes requires caspases activation and the intracellular accumulation of Fas in the MVB. Indeed, Fas activation triggers the endocytosis of Fas that strongly accumulates in a CD63-enriched compartment, corresponding to MVB. This accumulation in the MVB after 3 hours of FasL stimulation correlates with the maximal caspases activation, suggesting an involvement of this process also in the apoptotic signaling.

Altogether our data suggest that the intracellular post-endocytotic trafficking of Fas to the MVB promotes cell death signaling and is a prerequisite to the biogenesis of Fas-dependent Exosomes.

Our project aims at evaluating the impact of the secretion of EV controlled by Fas on tumor development and metastasis. Our objectives are: i- to characterize the biogenesis mechanism of Fas dependent-EV and ii- to determine the role of these EV in modulation of the immune response and cancer progression. Our project will pave the way to future clinical studies aiming to use Fas dependent-EV as a biomarker in cancer progression.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-33

13 *Plant biology* **SOULE Salomé** ISA

The root-knot nematode effector MiEFF12 targets the host ER quality control system to suppress immune responses and allow parasitism

Root-knot nematodes (RKN) are microscopic parasitic worms able to infest roots of thousands of plant species and causing massive crop yield losses worldwide. Within host roots, RKN induce formation of galls by redifferentiating five to seven root cells into giant and multinucleated feeding cells. These giant cells supply water and essential nutrients for nematode development. The formation and maintenance of giant cells is the result of an extensive regulation of the gene expression within targeted root cells and manipulation of key host functions. Secreted RKN effectors are key actors of this cellular reprogramming. They are mostly synthesized in the esophageal glands of the nematode and injected into the plant cells via the syringe-like stylet. Recent progress in nematode genomics and transcriptomics, has allowed identifying a large panel of RKN-specific effectors, conserved in the genome of the five main RKN species: M. enterolobii, M. arenaria, M. javanica, M. incognita and M. hapla, and notably produced during the parasitic stages. By performing in situ hybridization, we demonstrated specific expression of some genes, e.g. EFFECTOR12 (EFF12), in the esophageal glands of diverse RKN species. To decipher the role of these candidate effectors during parasitism, we are characterizing their plant targets in tomato. In planta, MiEFF12 localizes to the endoplasmic reticulum. RNAseq analyses of Arabidopsis roots ectopically expressing MiEFF12 revealed the contribution of the effector in modulating host immunity. Yeast two-hybrid and split luciferase approaches identified an essential component of the ER quality control system, as a MiEFF12 partner in tomato. Finally, silencing the plant target decreased N. benthamiana susceptibility to M. incognita infection.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-34

Physio - Electrophy **REDMAN Elisa** IPMC

Effects of IL-13 on airway epithelial cell populations by single-cell RNA sequencing in the context of asthma

The airway epithelium is a tissue lining the inside of the airways (nose, trachea and bronchi) and is the first line of defense against external aggressions. It is mainly composed of basal cells, mucus-secreting goblet cells and multiciliated cells. The coordinated beating of the hundreds of cilia located at the surface of the multiciliated cells allows the clearing of the pathogen-trapping mucus, in a process called mucociliairy clearance. In asthma, the airway epithelium is subjected to chronic inflammation and impaired regeneration, leading to remodeling, goblet cell hyperplasia decrease in multiciliated cells and. Interleukin-13 (IL-13), secreted by Th2 cells, is an important cytokine in the pathogenesis of the disease. The implication of the immune system in this inflammation is well characterized, however, the epitheliumspecific mechanisms of asthma are unclear, and whether multiciliated cells can transdifferentiate into goblet cells is still up for debate. To identify cell trajectories giving rise to the asthma-related population imbalance, differentiated primary human airway epithelial cells were treated with IL13. Epithelial remodeling was observed after eight days of treatment. In order to mimic recovery, we then subjected the cells to a wash-out period of two weeks, which resulted in an impressive restoration of the initial cell composition. To identify recovery trajectories and elusive cell type intermediates, we performed single-cell RNA sequencing after the IL-13 treatment and after the wash-out period. Single-cell RNAseq confirmed that IL-13 treatment leads to epithelial remodeling and identified cell type-specific IL-13 target genes, with for instance, SERPINB10 expressed in multiciliated cells, or PIGR expressed in secretory cells, including goblet cells. IL-13 stimulation induces both a decrease in the number of MCCs and their progenitors (deuterosomal cells), as well as a reduction in the amount of cilia present at the apical surface of each cell. A decrease in the multiciliated cell transcriptomic signature suggests that MCC decrease is not only due to apoptosis but is also due to a loss of multicellular identity.

ORAL COMMUNICATIONS

3rd year PhD Students

OC-35

Cancer / Physio **CLOT Charlène** iBV

Importance of the cellular sex during tumor formation

Charlène CLOT[(1)], Rénald DELANOUE[(1,*)] and Bruno HUDRY[(1,*)]

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The incidence and mortality of various cancers are associated with sex-specific disparities. Even though sex plays a crucial role in a variety of cancer, its impact on the processes leading to tumor formation is not well understood. In order to characterize how the sexual identity controls the susceptibility to tumors, we study an animal model in which we can easily generate genetically-induced sex-specific tumors, *Drosophila*. Indeed, invalidation of *Notch* in the adult intestinal stem cells (ISCs) leads to growth of massive tumors specifically in the female midgut. Factors and molecular mechanisms involved in this sex-biased tumor formation are not known. In *Drosophila*, the female sex determinant is the splicing factor Transformer (Tra^F), the master regulator of female identity. Targeted and ectopic expression of tra^F in males leads to "feminization" at the organism, organ or cell level. For instance, we could show that ubiquitous and ectopic expression of tra^F induces tumors in males, showing that the female sex determinant is key in tumorigenesis. However, specific "feminization" of either the ISCs or the whole intestine has no impact on tumorigenesis, suggesting that tra^F requirement is not cell or organ-autonomous. By "feminizing" different organs or cell types, we could finally determine that ectopic expression of tra^F in a neuronal population named *fruP1* is sufficient to induce massive tumorigenesis in male midguts. The same approach allowed us to show that ectopic expression of tra^F in the peptidergic neurons induces tumor formation. These data unravel an unexpected connection between the nervous system and the gut. This gives us the opportunity to identify the molecular actors linking peptidergic neurons to ISCs properties. *Drosophila* has only 50 neuropeptides. Therefore, we carried out a biased genetic screen by silencing their receptors in the ISCs in a tumor context. We obtained three candidates, CCAP, Tachykinin and Proctolin, that we are currently testing.

POSTER COMMUNICATIONS

2nd year PhD Students

P-1

Neurobiology **BOULSKIBAT Asma** IPMC

Fragile X syndrome: identification of a new drug candidate

Asma BOULKSIBAT, Sébastien DELHAYE, Marielle JARJAT, Maria CAPOVILLA, Alessandra TEMPIO, Barbara BARDONI

Fragile X syndrome (FXS) is a leading cause of inherited intellectual disability. Patients present a peculiar facial phenotype, socio-cognitive deficits, hyperactivity and seizures. In the brain of patients, abnormal dendritic spines have been described. This neurodevelopmental disorder is due to the loss of FMR1 gene expression leading to the absence of FMRP, an mRNA binding protein. No treatment is available for FXS. Therefore, identifying molecules for potential therapeutic approaches is a priority in the field. Using an FXS cell line model (shFmr1ECs), we carried out a screening of three libraries of small molecules at the 'Plateforme de Chimie Biologique Intégrative de Strasbourg' resulting in the selection of an FDA approved drug (SM4) targeting histamine receptors (HRs). To study further this drug, I used the FXS mouse model (Fmr1-KO) that recapitulates the main deficits of the disorder. First, I tested the acute administration of SM4 to Fmr1-KO mice and I observed a rescue of their socio-cognitive deficits. Remarkably, the chronic administration of the drug to infant Fmr1-KO mice rescued their hyperactivity in adulthood, proving the ability of SM4 to change the trajectory of the disease. Histological brain studies of these mice showed an improvement of the neuronal and microglial morphology. Altogether, these data encourage me to continue the project in order to better characterize HRs as targets to treat FXS since they were never previously involved in the pathophysiology of this disorder.

POSTER COMMUNICATIONS

2nd year PhD Students

P-2

Physio - Electrophy **VIVIER Charles-Maxime** IRCAN

Analysis of the genetic signature of regenerative epithelial skin cell population

Charles-Maxime Vivier, Laurence Cailleteaux, Soline Estrach, Chloé Féral

“Epithelial homeostasis and tumorigenesis” team

IRCAN

The skin is a complex organ undergoing crucial regeneration cycle ensuring maintenance of its function. The functions are protection, thermoregulation, and sensory organ. Skin contains 3 layers: the hypodermis, the dermis, and the epidermis (from inside out). The epidermis, itself, is constituted of 3 epidermal lineages: the interfollicular epidermis, the sebaceous gland and the hair follicle. The hair follicle is considered as a mini organ as it is subjected to its own regeneration cycle. The hair follicle represents a remarkable experimental model in the field of regeneration. Interestingly, the adult stem cell population fueling this regenerative process has been identified and characterized by the existence of many complementary and redundant markers. However, there is a strong heterogeneity of these markers, which could hide a putative hierarchy and even more a true reservoir population. To address this question, we performed a single-cell RNAseq analysis of murine follicle stem cell at D28, representing the activated pool of stem cells and unraveled the existence of several cell clusters usually hidden during the resting phase of the follicle. After validation of these clusters and bioinformatics analysis of the gene networks enriched in these clusters, we isolated one cluster that could be a candidate subpopulation for the reservoir. This cluster displayed a unique genetic enrichment supporting the maintenance of its stemness potential and was not previously described in the literature. To further characterize this population, we will now focus on validating the function of the new markers enriched in this population, via *in vitro* experiments, analyzing the cluster of interest behavior during aging and testing its regenerative potential via *in vivo* transplantation experiments.

POSTER COMMUNICATIONS

2nd year PhD Students

P-3

Plant bio **LUPATELLI Carlotta** ISA

Automated high-content image-based characterization of microorganism behavioral diversity and distribution

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Microorganisms, environment, exploration, automated image analysis, cell tracking

Microorganisms evolved complex systems to respond to environmental signals. Gradients of particular molecules alter microbe behavior and distribution within their environment. The development of a micrometric survey system could reveal microbe-environment interaction dynamics and improve our understanding of microbiota formation on host plants. Here, we provide a comprehensive step-by-step protocol for the characterization of species-specific behavior of a mixed microbial suspension. We coupled microfluidic technologies to automated high-content image analysis to morphologically discriminate three different telluric species (Phytophthora parasitica, Vorticella microstoma, Enterobacter aerogenes) and characterize their behavior response to a potassium gradient driver. Using TrackMate plug-in algorithms within Fiji, we conducted morphometric and motion analyses to elucidate the response of individual microbial species to the driver. The methodology adopted enabled us to confirm the different shape features of the three species and to simultaneously characterize their particular motion adaptation to the driver, so far unknown for V. microstoma and E. aerogenes as well as their co-interaction dynamics. The results obtained demonstrated the effectiveness of the method to screen complex microbial dynamics at high spatial and temporal scales. For more complex microbial suspension, the method can potentially be integrated in support of classic omics approach or as a screening strategy for biocontrol agents evaluation, enlightening possible beneficial-pathogenic interactions based on co-colonization of microhabitats.

POSTER COMMUNICATIONS

2nd year PhD Students

P-4

Developmental bio **VALERO Florian** iBV

The interplay between RNA-RNA interaction and RNA condensation

The translation control of mRNAs is a dynamic process that allows a rapid adaptation of gene expression in response to a stimulus. In the cytosol of eukaryotic cells, the translation of millions of mRNA is adapted to stress. How the translation of these large collective of transcripts is coordinated remains to be addressed. Recent results in the field have shown that repressed mRNAs can be found in three forms: (1) in the form of single mRNAs that are soluble in the cytosol; (2) in the form of homotypic clusters containing multiple mRNAs of same sequence identity, whose size is at the limit of classical optical resolution; (3) in the form of heterotypic macro-condensates containing thousands of mRNAs identities, and that can reach sizes greater than nuclei. The dynamics of these assemblies are regulated. The depletion of a helicase leads to the change of macro-condensates from a liquid-like to a solid-like form. During my thesis I propose to address the mechanism of repressed mRNA aggregation during the stress response and test the role of the regulatory proteins that control the underlying RNA-RNA interactions.

To address the first question, I developed a method to map the RNA-RNA interactions that drive RNA aggregation. Briefly, I synthesised *in vitro* various mRNA constructs that are fluorescently labelled to test whether they retain their aggregation capacity when injected in *C. elegans* gonad. For that purpose, I adapted a new method of labelling RNAs Between Body and Tail, that allows fluorescent labelling of RNAs without inserting modified nucleotides into the coding sequence and UTRs to preserve mRNAs secondary structures. To validate the construct functionality, I first confirmed that the synthetized mRNAs retained their ability to recruit endogenous repressors such as CAR-1 when injected. Following such a validation, I am currently assessing the aggregation ability of various mRNA constructs.

To address the second question, I inhibited the functionality of an RNA granule helicase, either by depleting it by RNAi or by using temperature sensitive loss of function mutations, and I tested the consequences on mRNA organisation within the granule using high resolution microscopy. Preliminary results suggest that inhibiting the helicase activity induces the mixing between mRNAs that were previously separated in distinct granule territories.

Addressing the mechanisms of granule assembly should allow me to test their functionality in stress adaptation.

POSTER COMMUNICATIONS

2nd year PhD Students

P-5

Cancer | **DURANDY Manon** | IRCAN

CD98hc as potential therapeutic target in KRAS-driven lung cancer"

Mots clés KRAS, CD98, lung, cancer

Durandy Manon, PhD 2ème année

Équipe « Homéostasie épithéliale et tumorigenèse », IRCAN

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Lung adenocarcinoma, the major form of lung cancer, is the deadliest cancer worldwide and exhibits pronounced inter- and intra-tumor heterogeneity cofounding precision medicine. KRAS is the most frequently mutated gene in lung adenocarcinoma. Its mutation correlates with tumor heterogeneity and leads to a poor prognosis.

Lung adenocarcinoma results from the transformation of several progenitor cells that accumulate genetic abnormalities. Stem cell maintenance is regulated through stem/progenitor cells and their niche dialog and a deregulation of these interactions leads to tumorigenesis. When mutated, KRAS leads to cellular reprogramming of the cell toward a 'stem' phenotype resulting in tumorigenesis. Thus, a better understanding of how genetic alterations and perturbed microenvironments impact progenitor-mediated tumorigenesis and treatment response is of the utmost importance to develop new therapeutic opportunities. Our results highlight that KRAS-driven tumorigenesis can proceed through both CD98hc-dependent and independent pathways depending on tumor cell-of-origin. CD98hc functions as amino acid transporter and co-receptor for integrins. CD98hc is involved in wound healing and its expression correlates with poor prognosis in lung cancer. The overall goal of my project is to understand the role of CD98hc in KRAS-driven lung cancer depending on tumor cell of origin.

POSTER COMMUNICATIONS

2nd year PhD Students

P-6

Biochemistry	ANGELELLI Francesco	C3M

Mir21 binding sites Interaction and Identification in hepatocytes

microRNAs (miRNAs) are ~22 nucleotide in length small noncoding RNAs that associate with Ago proteins to post-transcriptionally silence target RNAs by sequence pairing. More than 2000 miRNA species are present in human genome. They are fundamental players of gene expression control. The complexity of post-transcriptional repression is underlined by the occurrence of multiple miRNA-binding sites on the same RNA, which repression can result by either independent or cooperative action. In the context of post-transcriptional control, a combinatorial code between CIS (RNA sequence domains) and TRANS elements (miRNAs, but also RNA-binding proteins) enables a fine control of the fate of target RNAs, by masking or exposing specific binding sites. This is a relatively new and unexplored concept of gene expression control and its (de)regulation is involved in normal and pathological physiology. In my thesis, I aim at providing novel insights about the rules governing miRNA-binding sites interplay using molecular biology approach coupled to computational analysis. To this goal, I am using miR-21 in hepatocytes as pathophysiological model. miR21 is expressed in hepatocytes and its upregulation isinvolved in the pathogenesis of obesity and its associated comorbidities, including diabetes. Hence, the main hypothesis of my thesis is that upregulation of hepatic miR-21 may alter the post-transcriptional equilibrium between CIS and TRANS elements causing a deregulation of hepatocyte phenotype in obese patients. My Aims are: 1) to identify miR-21 binding sites in hepatocytes using the iCLIP2 technique; 2) to study the interplay between miR-21 binding sites to each other and other miRNA-binding sites by computational approach to trigger a greater repression of the targets, using the data generated by the iCLIP2 technique coupled with RNAseq (miR21 knockdown) in hepatocytes; 3) to validate such an interplay on selected miR21-targets by biochemical and molecular biology approaches; 4) to investigate to cell physiology impact of such a mechanism by bioinformatics (including IPA) and biochemical analysis (pathway signalling by western blot). In conclusion, my project will contribute to unfold the complexity of the miRNA-target repression and gain insights into the role of miR-21 in the liver pathophysiology.

POSTER COMMUNICATIONS

2nd year PhD Students

P-7

Immuno | **TEISSEYRE Maxime** | UR2CA

Impact of Pollution on the Pathophysiology of Membranous Nephropathy

Membranous nephropathy (MN) is a kidney autoimmune disease and a major cause of nephrotic syndrome. Its pathophysiology is complex and involves : (i) a deregulation of humoral immunity, with the production of autoantibodies; and (ii) a deregulation of cellular immunity, with an increased production of Th2 and Th17 cytokines. The incidence of MN has been shown to correlate with exposure to pollution, including fine particulate matter and organic solvents. In a previous study, we have shown that MN patients had higher levels of pro-inflammatory Th2 and Th17 cytokines than healthy controls. Patients with the highest levels of Th17 cytokines had the highest exposure to fine particulate matter. These patients also had a higher risk of disease relapse. Our hypothesis is that pollutants may redirect the cellular immune response towards the Th17 pathway. The objectives of this study were : (i) to investigate the role of fine particulate matter on the cytokine profile of MN patients; and (ii) to identify treatments that could be used to block the Th17 pathway. One milliliter of whole blood from MN patients or healthy donors was collected and exposed or not to fine particulate matter for eight hours at 37°C for the first objective or to different immunomodulatory treatments for two hours at room temperature for the second objective. Then the immune cells were stimulated with a QuantiFERON Monitor® tube (Qiagen) containing anti-CD3 as a T cell stimulant and R848 as an innate immunity cell stimulant. Stimulated blood samples were incubated for 16 to 24 h at 37°C and then centrifuged to harvest the stimulated serum. Levels of eight cytokines (IL-17A, IL-12p70, IL-4, IL-5, IL-1b, IFNa, IL-10, and IL-6) were measured using custom-designed Ella (ProteinSimple™) cartridges. We have shown that fine particulate matter has a pro-inflammatory effect and induces the Th17 pathway in MN patients. This effect is mediated via Toll-like receptors 2 and 4. Anti-IL17, interferon α and levamisole are effective treatments to block the Th17 pathway. These treatments may limit the risk of disease relapse in vivo.

POSTER COMMUNICATIONS

2nd year PhD Students

P-8

Neurobiology **VANDERSTEEN Clair** CoBTeK

Alzheimer's Early Detection in Post-Acute COVID-19 Syndrome: A Systematic Review and Expert Consensus on preclinical assessments

Clair Vandersteen1,2*†, Alexandra Plonka2,3,4,5†, Valeria Manera2,4,5, Kim Sawchuk6, Constance Lafontaine6, Kevin Galery7, Olivier Rouaud8, Noua Bengaied9, Cyrille Launay10, Olivier Guérin3,11, Philippe Robert2,3,4, Gilles Allali8, Olivier Beauchet7,12, Auriane Gros2,3,4

Introduction: The risk of developing a neurodegenerative disease, especially Alzheimer's disease (AD) in older adults, increasingly is being discussed in the literature on Post-Acute COVID-19 Syndrome (PACS). Remote digital Assessments for Preclinical AD (RAPAs) are becoming more important in screening for early AD, and should continue to be available for PACS patients, especially for patients at risk of AD. This systematic review examines the potential for using RAPA to identify impairments in PACS patients, scrutinizes the supporting evidence, and describes the recommendations of experts regarding their use.

Methods: We conducted a thorough search using the PubMed and Embase databases. Systematic reviews (with or without meta-analysis), narrative reviews, and observational studies that assessed patients with PACS on specific RAPAs were included. The RAPAs that were identified looked for impairments in olfactory, eye-tracking, graphical, speech and language, central auditory, or spatial navigation abilities. The recommendations' final grades were determined by evaluating the strength of the evidence and by having a consensus discussion about the results of the Delphi rounds among an international Delphi consensus panel called IMPACT, sponsored by the French National Research Agency. The consensus panel included 11 international experts from France, Switzerland, and Canada.

Results: Based on the available evidence, olfaction is the most sustained impairment found in PACS patients. However, expert consensus statements recommend that AD olfactory screening should not be used on patients with a history of PACS at this point in time. Experts recommend that olfactory screenings can only be recommended once those under study have reported full recovery. This is particularly important—for the olfactory identification subdimension. The expert assessment that more long-term studies are needed after a period of full recovery, suggests that this consensus statement requires an update in a few years.

Conclusion: Based on the available evidence, olfaction could be durably impaired in PACS patients. According to expert consensus statements, AD olfactory screening is not recommended for patients with a history of PACS until complete recovery has been confirmed by PACS olfactory recovery studies, particularly for the identification sub-dimension. This consensus statement may require an update in a few years.

POSTER COMMUNICATIONS

2nd year PhD Students

P-9

Bioinformatics | **OKOROKOVA Larisa** | IRCAN

How to functionally annotate mobile element insertions in human genome?

Larisa Okorokova PhD student at Gael Cristofari's lab "Retrotransposons and genome plasticity"

Individual human genomes show diverse array of genomic variations. One form of such variation comes from mobile element insertions. Mobile element insertions are created by LINE-1 retrotransposons, which are active in the human genome and can insert into new locations through a copy-and-paste mechanism. LINE-1 can also provide retrotransposition machinery to other, non-autonomous, transposable elements. Predicting the effect of mobile element insertions on phenotype is a complex task as most insertions occur outside genes or in intronic regions. Moreover, large-scale functional annotation of mobile element insertions faces the problem of limited amount of data – 1) populational dynamic of such polymorphisms is poorly described; 2) there are only anecdotal examples of polymorphisms associated with phenotype. We want to address this problem by creating machine learning framework which will assign an impact score to each mobile element insertion. Two main classes of features will be used in the framework – 1) features related to genomic location of insertion (presence of genes, regulatory elements, level of conservation, region mutability, etc.); 2) features related to internal sequence of insertion (presence of polyA signals, splicing acceptors, promoters, etc.). Our framework will help to prioritize newly discovered mobile element insertion depending on their potential impact and can be further used in the diagnosis of genetic diseases or cancers, including patients personalized therapies.

POSTER COMMUNICATIONS

2nd year PhD Students

P-10

Immuno / Physio	**MUCEL Inès**	C3M

p53 regulates macrophages phenotype and their capacity to handle lipids

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Université Côte d'Azur, INSERM U1065, Centre Méditerranéen de Médecine Moléculaire, Nice, France.

Metabolic reprogramming of adipose tissue macrophages (ATM) during obesity plays a major role in adipocytes dysfunction and contributes to the development of insulin resistance (IR) and Type 2 Diabetes. Transcription factors (TF) are crucial regulators of transcriptional programs involved in the polarization and adaptation of macrophages to their environment. Preliminary data showed an increase in the expression and activation of p53 in obese ATM. Thus, we decided to investigate the role of p53 in macrophages polarization, and its impact on adipocyte function. We used bone marrow-derived macrophages (BMDM) knockout (KO) for p53 and control BMDM, that we polarized into macrophages metabolically activated (MMe) with insulin, palmitate, and glucose, which phenocopy obese ATM phenotype.
We showed that macrophages polarized into MMe produced inflammatory mediators and expressed lipid handling genes. The invalidation of p53 potentiate the polarization into MMe with an upregulation of genes involved in lipid metabolism such as *Pparγ1, Cd36* and *Abca1*. Moreover, the cellular respiration was increased in p53 KO MMe compared to control MMe, suggesting a better oxidative capacity. In contrast, the activation of p53 before the polarization into MMe decreased the respiration compared to control MMe. Strikingly, compared to conditioned-media (CM) from control MMe, CM from p53 KO MMe did not alter insulin-induced PKB phosphorylation and the expression of insulin signalling genes in adipocytes. Moreover, adipocytes treated with CM from p53 KO MMe exhibited an upregulation of genes involved in lipogenesis.
In conclusion, our data suggest that activation of p53 in ATMs during obesity inhibits their ability to manage lipid and their oxidative capacity and contributes to the secretion of factors impairing insulin signalling in adipocytes. Thus, the dysregulation of p53 in macrophages during obesity could contribute to adipose tissue dysfunction and IR.

POSTER COMMUNICATIONS

2nd year PhD Students

P-11

Cancer | **BOUVET Océane** | C3M

Biomechanical reprogramming by DDR1/2 collagen receptors in cutaneous melanoma: Impact on metabolism and therapeutic response

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[3]*Plateforme d'étude du métabolisme, SFR Necker, Paris*

Cutaneous melanoma is an aggressive and invasive skin cancer that remains a clinical challenge despite major advances with targeted therapies (TT) and immunotherapies. Upon microenvironment and therapeutic pressures, melanoma cells can switch from a melanocytic differentiated state to dedifferentiated states associated with increased expression of receptor tyrosine kinases and mesenchymal markers (1-4). Such adaptive plasticity was described as a driver of resistance to TT. We previously described that TT-exposed dedifferentiated melanoma cells acquire mechanical properties and extracellular matrix (ECM) remodeling activities in vitro and in vivo, (5-7). Additional experiments showed that ECM signaling on melanoma cells involves the collagen receptors DDR1/2, which activate a NIK/NFkB2 pathway involved in drug resistance (7). Further analysis of melanoma cells cultivated on soft and stiff collagen matrices indicated that DDRs are involved in stiffness-induced proliferation and motility of dedifferentiated melanoma cells. However, the mechanisms of action of DDRs in melanoma mechanoresponses is still unknown. Here we found by metabolomic analyses a mitochondrial ß-oxidation and mitochondrial electron transport chain enrichment in dedifferentiated cells cultivated on stiff collagen matrices. Seahorse analyses further showed a loss of mitochondrial respiration in cells plated on soft collagen matrices. Mechanistically, we show that DDR2 mediates the activation of the NIK/NFκB2 pathway by mechanical signals. We also provide evidence that mechanical signals affect mitochondria dynamics of dedifferentiated melanoma cells through the kinase NIK, which phosphorylates DRP1, a dynamin-related GTPase playing a key role in mitochondria fission and fusion. Electron microscopy analysis of mitochondria network of cells cultivated on soft *versus* stiff collagen matrices confirmed the action of DDR2 on ECM stiffness-driven mitochondria fusion. Interestingly, exposure of melanoma cells to TT also leads to the modulation of mitochondria dynamics and increased fusion, suggesting that mitochondria integrate both extrinsic and intrinsic mechanical signals.

Together, these findings provide an original link between ECM-induced mechanical signaling, collagen receptors DDRs, melanoma cell metabolism and mitochondria dynamics. This study improves our understanding of the biomechanical cues from the tumor microenvironment that affect melanoma cell plasticity and adaptation to TT.

POSTER COMMUNICATIONS

2nd year PhD Students

P-12

Developmental bio **DETTI Mélanie** iBV

RNA methylation (m6A) in early gonadal development

During embryonic development, gonads are first bipotential, and have the ability to differentiate either into a testis or into an ovary in XY and XX embryo respectively. Upon signals which are partly identified, a proportion of somatic progenitor cells will differentiate into Sertoli cells or granulosa cells respectively, thus initiating the whole sexual development of the individual. In parallel, germ cells proliferate actively and then lose their pluripotency and enter meiosis in the ovary while they remain pluripotent and enter quiescence in the embryonic testis. In male mice, germ cells resume proliferation and enter meiosis after birth. The transition from proliferation to meiosis occurs through a change in the genetic program regulated by the somatic environment. However, the mechanisms controlling this change are still not fully understood.

The m^6A methylation is the methylation at position 6 of the adenine molecules of RNA and the most abundant of RNAs. It is a regulatory mechanism of gene expression whose dysfunctions promote different types of pathologies and cancers. The catalytic unit of methylation is the methyltransferase METTL3, which associates with METTL14 and promotes binding to a consensus RNA sequence. The WTAP protein associates with the METTL3/14 complex and promotes nuclear localization of the complex and recruitment of substrate. When m^6A methylation is deposited, "reader" proteins can alter RNA splicing, stability, or translation, allowing for a wide variety of processes such as metabolic, developmental, cellular differentiation, or stress response. This type of methylation is involved in fertility in many species like Drosophila, zebrafish, and mouse and meiosis entry in yeast. Although mutations in key genes involved in m^6A methylation lead to sex-independent infertility, detailed studies in mice have been performed only in males after birth.

My thesis project consists of deciphering the role of m^6A RNA methylation in the very early stages of germ cell and somatic cell differentiation using as an entry point the *Wtap* gene, an essential component of this molecular mechanism. My results suggest that *Wtap* is a new actor of sex determination in mice.

POSTER COMMUNICATIONS

2nd year PhD Students

P-13

Immuno / Microbio **FICHANT Arnaud** ISA

Characterization of the virulence potential of spore-forming Bacillus cereus in Drosophila melanogaster

Keywords: Bacillus cereus, Bacillus thuringiensis, foodborne outbreak, spores, Drosophila

Bacillus cereus sensu lato or Bacillus cereus group (Bc) is composed of Gram-positive, aerobic and facultative anaerobic spore forming bacteria. This group hosts opportunistic bacteria well known for their involvement in foodborne outbreaks (FBOs). In 2019, Bc represented the leading cause of FBOs due to bacterial toxins in Europe according to the European Food Safety Authority (EFSA)[1], triggering mainly gastrointestinal disorders such as diarrhea, vomiting and abdominal pain. Though the action mechanisms of the Bc diarrheal toxins (Nhe, Hbl and CytK) are beginning to be fairly well described in the literature [2], what is triggering the opportunism of some strains remains poorly understood. In agreement, EFSA has highlighted the lack of data needed to assess the pathogenic potential of Bc including Bacillus thuringiensis (Bt) [3]. Spores of Bt are commonly use as biopesticide, raising the question of potential effects on non-target organisms.

In this context and with the aim to better understand the virulence mechanisms of spore-bacteria belonging to the Bc group, we first performed an in silico analysis of the genomes of 37 relevant strains to identify potential virulence factors. Then, using Drosophila melanogaster (Dm) as oral infection model by spores, we assessed the in vivo virulence of the 37 strains. Interestingly, we have identified four virulence groups among these strains, ranging from high to no mortality. The early mortality was correlated with the loss of the intestinal barrier integrity. Finally, WGS analysis allowed the identification of a large number of virulence factors potentially involved.

This work will contribute to a better understanding of the pathogenic mechanisms associated with the opportunism of Bc following spore ingestion.

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POSTER COMMUNICATIONS

2nd year PhD Students

P-14

Physio - Electrophy / Neuro **SIMONTI Martina** IPMC

A novel mechanism of non-syndromic Autism Spectrum Disorder caused by mutations of the SCN2A gene: functional studies and knock-in mouse model.

Simonti M., Cestèle S., Léna I., Mantegazza M.

Mutations in the SCN2A gene, encoding the voltage-gated sodium channel Nav1.2, cause a wide range of phenotypes, including different types of epilepsy, intellectual disability, schizophrenia and autism spectrum disorder (ASD). ASD is a neurodevelopmental disorder characterized by social communication deficits, impaired social behavior and stereotypies. ASD-associated SCN2A variants induce a loss of Nav1.2 function, determining haploinsufficiency in heterozygosis, but the pathological mechanism is not clear yet. A functional study carried out by our team showed that the mutations found in patients with non-syndromic ASD specifically induce a negative dominance in HEK cells and neurons co-expressing WT and mutant channels, leading to an overall >50% reduction of functional Nav1.2 (article in preparation). We generated a new heterozygous knockin (KI) mouse carrying one of these mutations, Scn2aL1314P. 1) Using patch-clamp recordings in brain slices obtained from pups (P5-P9) and young mice (~P25), we are evaluating potential impairments in the medial prefrontal cortex (PFC) layer 5 pyramidal neurons (PYRs) caused by the mutation in Scn2aL1314P/+ mice. 2) To determine how these alterations may affect the phenotype, we are characterizing mice behaviour through a battery of tests, meant to assess ASD core symptoms and comorbidities at young and adult age.

POSTER COMMUNICATIONS

2nd year PhD Students

P-15

Cancer / Immuno **MOUSSET Alexandra** IRCAN

Role of Neutrophil Extracellular Traps (NETs) in squamous cell carcinoma (SCC) development

Inflammation is a natural response of the immune system to injury or infection, aimed at removing harmful stimuli and initiating tissue repair. However, chronic inflammation is known to predispose to cancer development as it creates a favorable environment for all step of carcinogenesis[1] . Conversely, cancer cells, similar to tissue injury, can trigger an inflammatory response to promote cancer progression[1]. However, the cellular and molecular mediators of inflammation modulating cancer initiation and progression are unclear. In this context, immune cells have been studied to understand the role of inflammaton in cancer development. Even though neutrophils constitute the largest percentage of leukocytes in blood and that they are among the first cells to arrive at the inflammatory site, it is only recently that their role in cancer has attracted attention[2] . Outside of cancer, neutrophils can kill harmful microorganisms via (i) phagocytosis; (ii) degranulation of cytotoxic enzymes and proteases; or (iii) formation of neutrophil extracellular traps (NETs). NETs are DNA strands associated with enzymes and proteins and released in the extracellular space to trap and kill pathogens during infection. Recently, we and others determined that NETs have pro-metastatic activities[3-6] and that they can protect metastatic cancer cells from chemotherapy[7] . However, the role of NETs in all step of carcinogenesis is still unclear. To study the role of NETs in all step of cancer development we use the DMBA/TPA model of cutaneous squamous cell carcinoma (cSCC). Our results show that targeting NET-forming neutrophils counteract significantly the development of cSCC in vivo. In addition, using in vitro 3D models, we show that NETs promote the proliferation of SCC cells. These results suggest that NETs participate in all the different step of cSCC progression. Accordingly we show that NETs are present in 70% of SCC biopsies analyzed. We now try to understand the underlying mechanism using both in vitro and in vivo strategies. Our hypothesisisthat NETs 1) increase DNA damage in keratinocytes, leading pro-cancerous mutations accumumation; 2) promote an anti-apoptotic response in the damaged keratinocytes; 3) promote keratinocyte proliferation. Overall, our work aims at identifying the role of NETs in the initiation and progression of SCC, which we hope will lead to the emergence of new therapeutic targets.

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POSTER COMMUNICATIONS

2nd year PhD Students

P-16

Physio - Electrophy **TOFT Maurizio** IPMC

ASIC1a channels participate to pain processing by dorsal spinal cord neurons

Maurizio Toft, Ludivine Pidoux, Magda Chafai, Kevin Delanoe, Perinne Inquimbert, Emmanuel Deval

Dorsal horn of the spinal cord is a key point of the pain neuraxis where sensory-nociceptive informations, coming from the periphery, enter the central nervous system to be integrated, processed, and sent to the brain. Spinal inputs come from peripheral Aβ, Aδ and C fibers and, importantly, these inputs can be subject to different facilitation/sensitization processes, leading to pain hypersensitivity and allodynia. Among these processes, windup is a “short term” facilitation of C-fiber inputs following peripheral low-frequency repetitive stimulations, resulting in a progressive increase of the number of action potentials (APs) evoked by wide dynamic range (WDR) neurons. Windup is therefore an interesting way to study the processing of nociceptive information by spinal cord neurons. Moreover, although windup was initially described several decades ago, its exact mechanism is still not fully understood.

Here, we combine both in vivo and ex vivo electrophysiological recordings to demonstrate a role for ASIC1a channels in the windup process. Pharmacological inhibition of spinal ASIC1a channels by different venom peptides (PcTX1 and Mambalgin-1) lead to significant decreases of the windup process, and we characterize a native ASIC current in WDR neurons. The kinetics and pharmacological sensitivities to this native current suggest the functional presence in WDR neurons of ASIC1a channels, either in homomeric or heteromeric (i.e., ASIC1a/ASIC2a, ASIC1a/ASIC2b) forms. We next characterized the properties of these different ASIC1a channel subtypes in a heterologous expression system, and we show that the native ASIC current matches the properties of ASIC1a homomeric channel.

This work indicates that ASIC1a homomeric channels in WDR neurons of the dorsal spinal cord positively participate to the pain facilitation process of windup.

POSTER COMMUNICATIONS

2nd year PhD Students

P-17

Microbiology **PLUMB Emily** iBV

Tolerance to an Antifungal drug is Associated with Biophysical Changes to the Cytoplasm of a Human Fungal Pathogen

Emily Plumb, Antonio Serrano, Martine Bassilana, Robert A. Arkowitz

Université Côte d'Azur, CNRS, INSERM, Institute of Biology Valrose (iBV), Parc Valrose, Nice, France.

Antifungal drug resistance occurs when a genetic advantage enables survival in otherwise inhibitory concentrations. However, in many clinical isolates, a portion of the cell population exhibits slow-growth upon prolonged drug exposure above the minimal inhibitory concentration (MIC) [1, 2]. This subpopulation, referred to as tolerant cells, has been linked to recurrent and persistent fungal infections, such as those due to the opportunistic fungal pathogen *Candida albicans.*

We are investigating the consequences of prolonged exposure to supra-MIC antifungal drugs, including the widely used fungistatic drug fluconazole, on the biophysical properties of the *C. albicans* cytoplasm. Fluconazole targets an enzyme in the ergosterol biosynthesis pathway; this sterol is a critical lipid in the fungal plasma membrane. To examine cytoplasmic crowding/viscosity, we have optimized a micro-rheological probe, taking advantage of dynamics of genetically encoded multimeric nanoparticles (GEMs) [3]. Initial results indicate an increase in cytoplasmic crowding upon prolonged exposure to supra-MIC fluconazole, conditions associated with drug tolerance. These effects were observed after 7 hours and were maximal after 1 day, despite substantial cell-to-cell variation in GEM effective diffusions. As previously reported, in the presence of high fluconazole concentrations, aberrant cell morphologies were observed [4], however there does not seem to be a correlation between these specific morphologies and increased cytoplasmic crowding/viscosity. To ascertain that this effect of fluconazole on the biophysical characteristics of the cytoplasm are due to the drug inhibition of its target, the Lanosterol 14-alpha-demethylase Erg11, we carried out complementary studies in *erg11* mutants. Initial results, similarly, reveal an increase in cytoplasmic crowding/viscosity upon reduced expression of *ERG11*. In conclusion, specific inhibition of ergosterol biosynthesis, by both antifungal drug treatment and genetic manipulation, dramatically increases cytoplasmic crowding/viscosity consistent with the attractive possibility that cytoplasmic phase transitions play a critical role in antifungal drug tolerance.

POSTER COMMUNICATIONS

2nd year PhD Students

P-18

19 *Biochemistry / neuro* **MINNITI Julien** IPMC

PARKIN TRANSCRIPTIONNALY REGULATES iNOS IN MICROGLIAL NEUROINFLAMMATION

Mutations in the PRKN gene encoding Parkin (PK) are autosomal recessive. They are the most common mutations found in juvenile Parkinson's disease (PD). In addition to its canonical function as an E3-ubiquitin ligase (Ubi), Parkine also acts as a transcription factor (TF). Through its two functions, PK regulates the expression of genes associated with pathologies such as brain cancers or neurodegenerative diseases. Some studies document a correlation between increased neuroinflammation (NIF) and the absence of Parkine. Our objective is to determine the involvement of the TF activity of PK in neuroinflammatory processes.

Following preliminary results on the impact of PK in NIF, we focused on the inducible-NO synthase (iNos). The characterization of iNos was analyzed by RTqPCR, western blot and nitric oxide fluorometric assay. The impact of PK on iNos was measured by western blot and RTqPCR. The transcriptional activity of NFκB-p65 and the promoter activity of iNos was performed by fluorometric assay of luciferase activity.

In this study, we demonstrate that PK overexpression induces iNos transcription during acute inflammatory stress generated by lipopolysaccharide (LPS) treatment of murine microglial cells (BV2). Indeed, overexpression of PK increases the level of protein and mRNA expression of iNos during LPS stress. Interestingly, PK also increases the transcriptional activity of NFκB-p65 which is itself known as a TF of iNos. However, by using a specific inhibitor of NFκB-p65, we retain the regulation of iNos by PK. Our results therefore indicate an effect of Parkin on iNos expression independent of NFκB-p65 action. Finally, PK increases the promoter activity of the iNos gene independently of LPS suggesting a direct TF regulation of this gene.

Thus our work unmasks transcriptional control of iNos by PK independent of the Ubi function of PK and NFκB-p65. The regulation of the iNos promoter by PK reveals LPS independence. However, physiologically, the inflammatory stress activating NFκB-p65 is necessary for iNos transcription. Therefore, Parkin would be a modulator of iNos in microglial neuroinflammation. Our project would constitute a significant step forward to the understanding of PK role in the control of NIF in PD pathological context. The elucidation of the mechanisms underlying parkin transcriptional factor regulation of NIF mediators should open new avenues in PD therapeutic and biomarker research.

POSTER COMMUNICATIONS

2nd year PhD Students

P-19

Immuno	CHAFIK Abderrahman	C3M

Control of mitochondrial functions by endosomal GTPase Rab4bdependent mechanisms

Abderrahman Chafik1 , Lucien Daunas2 , Nathalie Mazure3 , Jean-Marc Verbavatz2 , JeanFrançois Tanti1 , Jérôme Gilleron1 , Mireille Cormont1

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Our team demonstrated that decreased expression of Rab4b, an endosomal Rab GTPase, in T cells impaired glucose and lipid homeostasis by favoring Th17 differentiation at the expense of Treg (Gilleron et al., Cell Rep 2018). Our goal is to understand how Rab4b controls the balance between Th17 and Treg differentiation. The differentiation process into Th17 and Treg relies on the capacity of naïve T cells to shift their metabolism to glycolysis for Th17 or oxidative phosphorylation for Treg. We made the hypothesis that Rab4b, by controlling mitochondrial functions in naïve T cells, determines their fate. We, therefore, want to 1) characterize the mitochondrial dysfunction due to the lack of Rab4b; 2) identify the Rab4b dependent processes responsible for these mitochondrial dysfunctions. To address these objectives, we use mouse primary CD4+ T cells purified from the spleen of Rab4bTcell KO mouse or their control littermates. Using seahorse, we found that the basal respiration with glutamine or glucose as substrates is lower in T cells lacking Rab4b. Maximal respiration is decreased as is mitochondrial ATP production. The acidification of the medium, a reflection of glycolysis, is also lower in Rab4binvalidated T cells. The number of mitochondria determined by electronic microscopy is unchanged. We detected a reduction by ~20% of the mitochondrial activity in Rab4binvalidated T cells with no change in mitochondrial mass using fluorescent probes, and this without an impact on viability. Rab4b-invalidated T cells have thus lower metabolic rates than control T cells. We wanted to confirm these results at a single-cell resolution using the flow cytometry-based approach SCENITH which is based on the postulate that translation level is reflecting ATP content. We found that the basal level of translation in Rab4b-invalidated T cells is unchanged compared to control cells, thus indicating that metabolism in Rab4b-invalidated T cells is sufficient to sustain protein synthesis. To understand how Rab4b tunes mitochondrial functions, we search by RNAseq for genes differentially expressed. We found genes implicated in OxPhos, and in Coenzyme A and Glutamine metabolism. We are also investigating by electron microscopy the impact of the down-modulation of Rab4b in T cells on mitochondrial morphology, on connections between mitochondria and endosomes. Our results indicate that Rab4b plays a role in the metabolism of T cells, the mechanisms involved remain to be established.

POSTER COMMUNICATIONS

2nd year PhD Students

P-20

Cancer | **KUNZ Sarah** | IRCAN

Nanobodies anti-CD98hc : Strategy against non-small cell lung cancer

Non-small cell lung cancer (NSCLC) is the leading cause of cancer deaths worldwide and the KRAS mutation remains the most common oncogenic driver in patients conferring a poor prognosis. One idea of personalized cancer therapy is to identify specific subgroups of cancer patients that will benefit from specific therapeutic strategies. Despite intensive efforts, targeted treatment options for KRAS-driven lung adenocarcinoma remain challenging. This is, in part, due to activation of alternative pathways, tumor heterogeneity and limited knowledge of RAS partners.

CD98hc, by interacting with integrins including integrin β3, regulates their function and modulate the cell microenvironment. Our preliminary results highlight CD98hc as a new putative target required for integrin β3/KRAS interaction. To date, CD98hc function cannot be compensated by another protein or alternative pathways identifying this protein as an ideal therapeutic candidate. Our preliminary results demonstrate that CD98hc genetic inhibition not only decrease KRAS-driven lung cancer initiation and progression but also prevents Integrin β3/KRAS interaction leading to KRAS-mediated downstream signaling inhibition. The goal of my project is to investigate the potential of CD98hc as therapeutic target for KRAS-driven lung cancer as well as provide novel pharmacologic inhibitors in the form of nanobodies.

POSTER COMMUNICATIONS

2nd year PhD Students

P-21

Physio - Electrophy | **CONTU Laura** | iBV

Role of thromboxane A2 in the browning of white adipocytes

The adipose tissue (AT) has a fundamental role in the gesture of energy resources. There are two types of AT : the white AT, specialized in storing and releasing lipids, and the brown AT, specialized in producing heat (thermogenesis). The activation of brown AT has beneficial effects on metabolism and insulin resistance, and favoring its formation and activation is proposed as a strategy to fight obesity and its comorbidities. Within the white AT, thermogenic adipocytes, called “brite adipocytes”, can appear under some conditions such as cold exposure, through the differentiation of dedicated stem cells, or through a conversion of existing white adipocytes. Understanding the processes regulating this conversion is of high interest. This process can be triggered in vitro by activating the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ), which controls the expression of several genes involved in the thermogenic phenotype, especially the Uncoupling Protein 1 (UCP1), the main marker of brite/brown adipocytes.

The purpose of this work was to analyze the role of the Thromboxane A2 (TXA2), an arachidonic acid metabolite, in the control of brite adipocyte formation and activation, using a human cell model (hMADS) and mouse models of obesity (genetic and nutritional).

hMADS cells are able to differentiate into white adipocytes and to subsequently be converted in brite adipocytes using rosiglitazone, a PPARγ agonist. We show that various amounts of two stable analogs of TXA2, CTA2 and U46619, inhibited, in dose dependent manner, UCP1 mRNA and protein expression during white to brite conversion process. The downregulation of UCP1 did not rely on a downregulation of PPARγ mRNA levels, but could rely on an inhibition of PPARγ activity through an inhibitory phosphorylation by the kinase ERK. While the phosphorylation of PPARγ upon TXA2 treatment remains to be analyzed, we show that TXA2 treatment increases ERK phosphorylation. Furthermore, we show that TXA2 not only inhibits the browning of white adipocytes but it favors the whitening of brite adipocytes. The effects of TXA2 target adipocytes, as treatment during the differentiation process did not affect the rosiglitazone-induced browning. Our data were validated using other cell models such as primary human and mouse cultures. Experiments using mouse models are in progress. These data point the TXA2 as a regulator of the whitening/browning process in adipocytes, and pave the way to develop new therapeutic tools to fight obesity and associated disorders.

POSTER COMMUNICATIONS

2nd year PhD Students

P-22

Neurobiology **ROYON Léa** IPMC

Control of motivated reward and aversion behaviors by the laterodorsal tegmental nucleus

A common trait of psychological disorders is an impairment in executing appropriate action under conflicting situations. Indeed, balancing reward and risk is a fundamental brain process essential for survival. The brain assigns positive or negative value to external stimuli, facilitating approach or avoidance behaviors, respectively. Thus, dissecting the brain circuitry that controls these processes is essential towards a better understanding of brain function and the pathological processes in mental disorders.

The laterodorsal tegmental nucleus (LDTg) is a heterogeneous brain region which modulates the reward system. However, recent work from our laboratory showed its implication in processing aversion. Thus, it is presently unclear what are the circuit mechanisms behind this diverse salience processing. Here, we investigate the role of the LDTg cholinergic neurons in a risk-taking paradigm in which mice have to balance the benefit of gaining a food reward with the cost of exposure to an aversive event. Using in vivo calcium imaging in transgenic mice, we found an increased activity of cholinergic neurons during the aversive event. Interestingly, this activation remains present during the extinction behavior, when no aversive event is presented. Moreover, we used optogenetics tools to modulate the cholinergic neurons of the LDTg during the risk-taking paradigm. We found that a specific inhibition of these neurons increases the risk-taking behavior of these mice.

Taken together, our study aims to undercover a new pathway that may contribute to the execution of appropriate action under conflicting situations.

POSTER COMMUNICATIONS

2nd year PhD Students

P-23

Plant bio | **NJEKETE Cliven** | ISA

Screening for nematicidal plants for root-knot nematode management in tomato agrosystems

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Biocontrol plants (BP) can be used in cropping systems to control pests directly by repelling, trapping, and killing or indirectly via promoting their natural enemies to act on the pest [1]. When BP are used as nematicidal plants (NP) to control root-knot nematodes (RKN), they could be non-host or poor host (allow penetration of RKN but starve them and prevent their reproduction, thus trap). This is accomplished by different modes of action: compounds found within the plant roots which are toxic to juveniles (J2) or compounds exudated in the soil that inhibit egg hatching and are lethal to J2 or modify their behaviour through repellence or attraction [2]. Two assays were conducted in climate chambers (n= 6+6, control: susceptible tomato) to test the effectiveness of 21 summer and winter NP candidates against Meloidogyne incognita Moreles and M. arenaria Avignon. Month-old seedlings were inoculated with 2000 eggs and analysed after one cycle for galls and egg masses on the roots and J2 in the soil. Tagetes spp. tested were non-hosts of both Meloidogyne spp., suggesting that the RKN barely penetrates the roots or reach its central cylinder to initiate their feeding sites. Significant varietal effects and different responses to the two species of Meloidogyne were observed for the other plants. Phacelia tanacetifolia and Avena strigosa were non-hosts of M. arenaria. The same was for Foeniculum vulgare cv. Rondo, Fagopyrum esculentum, and Crotalaria spectabilis on M. incognita. The rest were poor hosts of M. incognita and M. arenaria. In sorghums, if no or few galls were seen, egg masses were found inside the roots as already seen in some previous work [3]. Post one cycle, Meloidogyne J2s were found in the soil except in non-host plant modalities. Tagetes, Fagopyrum, Phacelia, and Foeniculum seemed the most "sanitising". Further, a complementary M. incognita J2 penetration assay was done on T. patula, T. erecta, F. vulgare, Crotalaria juncea, and tomato as control. C. juncea had more M. incognita J2 penetrations than tomato showing its affinity to attract and trap. Conversely, all the Tagetes spp. had lower penetrations than tomato and Crotalaria, probably due to repellence or toxicity. There is potential, guided by the different mechanisms, for all the tested BP to be in-cooperated and designed into cropping systems for RKN management. Keywords: Meloidogyne sp.; nematicidal plants, biocontrol plants; poor host plant, non-host plant

POSTER COMMUNICATIONS

2nd year PhD Students

P-24

Immuno / Microbio **AIEM Elody** Micoralis

Role of viral etiopathology in obstructive sleep apnea syndrome in children : case-control study

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Obstructive sleep apnea (OSA) is a common and widespread form of sleep-disordered breathing in children affecting from 1% to 5% aged 2–8 years [1,2]. It is characterized by repeated partial or full obstruction of the upper respiratory tract during sleep with episodes of apnea and hypopnea. Cardiovascular, neurocognitive and behavioral morbidities are associated with OSA [3–5]. Indeed, OSA enhances the risk of impaired cognitive function [6], attention deficit hyperactivity disorder [7], poor quality of life [8], enuresis [9], metabolic syndrome and cardiovascular disease [10]. For this reason, early diagnosis and adequate care are required to prevent long-term complications.
Palatine tonsils (PT), as secondary lymphoepithelial organs, are fundamental for immune responses to respiratory pathogens and allergic antigens. It is also known that some viruses, such as Epstein–Barr virus (EBV), human herpes virus 6, human immunodeficiency virus, measles virus and enterovirus, can contaminate the adenotonsillar tissues (AT) after an upper airway infection and may lead to persistent infection in an asymptomatic form during asymptomatic states [11–15]. Viral infections are prone to causing an inflammatory tonsil response leading to AT hypertrophy, which is by far the most important pathophysiological factor of OSA in children [16,17]. Right now, tonsillectomy and adenoidectomy remain the most effective and proposed treatment for children's OSA [18]. Nonetheless, the pathogenesis of AT hypertrophy leading to OSA in early childhood is poorly understood.

The protocol is a case-control study (R04-020, MR 1913210220). The objective is to compare the herpes-like viral present in children with and without SAOS, in order to propose a specific oral microbiological signature for OSA. The target populations are nine viruses of the herpes group, including HSV-1, cytomegalovirus (CMV), and EBV. A classic qPCR and RT-qPCR technique were carried out on the PT recovered within the department O.R.L. et de chirurgie cervico-faciale des Hôpitaux Pédiatriques de Nice CHU-LENVAL, then transferred to laboratory Microbiologie Orale, Immunothérapie et Santé UPR7354, University Côte d'Azur (UCA). The preliminary results obtained (32 cases *versus* 19 controls) following the identification by qPCR show that the presence of VZV, CMV and HHV-8 seems to be linked to OSA. Paves the way for further studies on the etiopathology of OSA, high-throughput Sequencing in collaboration with the Institut de Pharmacologie Moléculaire et Cellulair (UCA).

POSTER COMMUNICATIONS

2nd year PhD Students

P-25

Cancer / Physio | **TROJANI Marie-Charlotte** | TIRO/MATOs

AUTOPHAGY IS DESCREASED IN BONE OF OSTEOPOROTIC PATIENTS

Osteoporosis (OP) is a skeletal pathology characterized by major bone fragility that affects one out of 3 women after menopause. In addition to estrogen loss, an increase in oxidative stress is now recognized as a central mechanism in this pathology. Autophagy is an intracellular process allowing the removal and recycling of damaged proteins and organelles. Thanks to the elimination of damaged mitochondria, which are the main sources of reactive oxygen species, autophagy plays a major role in reducing oxidative stress. An age-related autophagy decline, associated with various experimental arguments in animal models, suggests autophagy implication in OP. However, a definitive proof in humans remains to be obtained.

In this work, we have analyzed autophagy by Western blot in bone samples from postmenopausal women with OP fracture, compared to controls undergoing total hip replacement for osteoarthritis. Our results indicate that the expression level of the autophagosome marker LC3-II is significantly decreased in OP patients compared to postmenopausal controls. In addition, the expression of the hormonally up-regulated neu-associated kinase (HUNK), which expression is upregulated by female hormones and promotes autophagy, is also significantly reduced.

Taken together, these data demonstrate for the first time that OP patients have a deficit in osteocyte autophagy and suggest that the HUNK kinase could be the factor linking estrogen loss and autophagy decline. This study will contribute to better understand the role of autophagy in OP pathophysiology and should lead to the design of new therapeutics targeting autophagy for the management of pathological bone aging.

POSTER COMMUNICATIONS

2nd year PhD Students

P-26

27 *Neurobiology* **FREMONT Gwendoline** IPMC

The role of parkin transcription factor function in neuroplasticity and memory formation in Alzheimer's disease

Gwendoline Fremont supervised by Dr. Alves da Costa, IPMC (Valbonne)

Parkin (PK), discovered in 1997, is a protein linked to Parkinson's disease (PD). The gene mutation leads to Parkinsonian syndrome with autosomal recessive transmission. This enzyme is encoded by the PARK2 gene, located on human chromosome 6. PK is composed of a Ubl domain, a RING-IBR-RING RING domain which gives it its ubiquitin ligase function. Interestingly, our team has demonstrated that PK is also a transcription factor that can regulate key genes such as those involved in PD, brain cancers, and Alzheimer's disease (AD). In addition, several articles show the involvement of PK in AD. A link has been described between PK and various molecular actors associated with the etiology of AD such as the TAU protein, the Ab peptide, and the g-secretase. Importantly, work by our team has demonstrated a contribution of PK transcriptional function in AD. Indeed, PK regulates the transcription of presenilins 1 and 2, two major components of the g-secretase complex involved in the production of senile plaques. Besides, PK, a pleiotropic protein, has been the subject of several studies giving it many functions such as a role in apoptosis, as a tumor suppressor, in mitophagy, and neuroplasticity. Referring to the neuroplasticity function, PK gene knockout mice (KO-PK mice) show alterations in dopaminergic neurotransmission and glutamatergic synaptic transmission in the hippocampus. Also, my team has demonstrated that PK regulates the transcription of XBP1, involved in memory formation, suggesting a role for PK in neuroplasticity. Regarding these data, we hypothesized that the transcriptional function of PK is involved in memory formation in AD. This hypothesis is supported by RNAseq results obtained with Nanostring's Neuropathology kit and 10- month-old KO-PK mice in a specific region of the brain that plays a fundamental role in learning and memory, the hippocampus. This experiment allowed us to discover a significant regulation of many genes involved in neuroplasticity. First, we confirmed these results by Q-PCR. Then we tested different cell models to confirm our preliminary results on SH-SY5Y human cells and primary cultures of neurons from PK-KO mice. Meanwhile, we characterized the neuroplasticity in KO-PK mice through the analysis by confocal microscopy of dendritic spines and synaptic buttons. To summarize, we propose to find common denominators between the familial form of Parkinson's disease and Alzheimer's disease to clarify the origins of these diseases.

POSTER COMMUNICATIONS

2nd year PhD Students

P-27

Physio - Electrophy **BIED Marion** iBV

Light Activation of Two Pore Domain Potassium Channels

Marion Bied, Brigitte Wdziekonski, Guillaume Sandoz

2-pore domain potassium (K2P) channels form a channel family traditionally known to be responsible for the leak potassium current in cells. Nevertheless, even though they are not voltage dependent, K2P channels are finely modulated by a wide range of stimuli, including mechanical forces, temperature and lipids, depending on the subfamily they belong to. Our work here focuses on another external cue: the light. We found that a pulse of light induced a strong and irreversible increase of the current of one of the mouse K2P members in both a duration and intensity dependent manner. We addressed the species specificity of this regulation and found that light-regulation is conserved in several species, but is not universal. Using chimeric and single point mutation approaches, we have been able to determine precisely the structure of the channel and amino acid involved. Together, our finding sheds light into the identified site's importance in K2P gating and a potential function of the channel in light sensing. Finally, physiological relevance of this new light gating mechanism is still under investigation.

POSTER COMMUNICATIONS

2nd year PhD Students

P-28

Plant bio / Omics **BENJAMIN Goodluck** ISA

NITROGEN-FIXING BACTERIA INDUCE DEFENSE PRIMING AGAINST PEA APHID IN MEDICAGO TRUNCATULA

Goodluck BENJAMIN1*, Marie PACOUD1, Stéphanie BOUTET2, Gilles CLEMENT2, Gregory MOUILLE2, Renaud BROUQUISSE1, Jean-Luc GATTI1, Pierre FRENDO1, Marylène POIRIÈ1 1 Université Côte d'Azur, INRAE, CNRS, Institut Sophia Agrobiotech, France 2 Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000 Versailles, France

Legumes form symbiotic relationships with nitrogen-fixing bacteria, allowing growth in nitrate deficient soils. Legumes are also prone to attacks by herbivores such as the pea aphid (Acyrthosiphon pisum). Beside their nutritional interests, plant-microbe symbioses can induce plant systemic defence reactions against bioagressors. The goal of this work is to study whether nitrogen-fixing symbiosis (NFS) can prime plant defence against pea aphid Acyrthosiphon pisum in the leguminous Medicago truncatula. We analysed the expression of defence genes and metabolite modification in both NFS and nitrate-fed (non-inoculated; NI) conditions with/without aphid infestation. Gene expression analysis showed that plants infested with aphids had significantly higher expression of Pathogenesis Related Protein 1 (PR1), a gene marker of the salicylic acid (SA) pathway, in both NFS and NI conditions. Proteinase Inhibitor (PI), a gene marker of the jasmonic acid (JA) pathway, was also induced by aphid infestation but with significantly higher expression in NFS conditions compared to NI conditions. GC-MS and LC-MS/MS metabolomics showed that 190 metabolites such as salicylate, pipecolate, gentisic acid and various cyclitols and phenols were significantly accumulated upon aphid infestation in the different plant feeding conditions confirming that aphid induced plant defence. Significant accumulation of 20 metabolites in NFS conditions compared to NI conditions suggests a possible immune priming effect on the plant defence by the symbiotic bacteria.

POSTER COMMUNICATIONS

2nd year PhD Students

P-29

Neurobiology **LANDES-CHÂTEAU Cassandre** UR2CA

Demyelinating lesions identification and McDonald criteria application facing white matter lesions on brain MRI

Introduction: White matter lesions (WML) on magnetic resonance imaging (MRI) are common in clinical practice. When analyzing WML, radiologists sometimes propose a pathophysiological mechanism to explain the observed MRI abnormalities, which can be a source of anxiety for patient. In some cases, discordance may appear between the patient's clinical symptoms and the identification of the MRI-appearing WML, leading to extensive diagnostic work-up. To avoid misdiagnosis, the analysis of WML should be standardized, and a consensual MRI reading approach is needed.

Objective: To analyze the MRI WML identification process, associated diagnosis approach, and misinterpretations in physicians involved in WML routine practice.

Methods: Through a survey distributed online to practitioners involved in WML diagnostic work-up, we described the leading causes of MRI expertise misdiagnosis and associated factors: clinical experience, physicians' subspeciality and location of practice, and type of device used to complete the survey. The survey consisted of sixteen T2-weighted images MRI analysis, from which ten were guided (binary response to lesion location identification), four were not shown (multiple possible answers), and two were associated with dissemination in space (DIS) McDonald criteria application. Two independent, experienced practitioners determined the correct answers before the participants' completion.

Results: 364 participants from the French Neuro Radiological (SFNR), French Neurological (SFN), and French Multiple Sclerosis (SFSEP) societies completed the survey entirely. According to lesion identification, 34.3% and 16.9% of the participants correctly identified juxtacortical and periventricular lesions, respectively, whereas 56.3% correctly identified non-guided lesions. The application of the 2017 McDonald's DIS criteria was correct for 35.3% of the participants. According to the global survey scoring, the factors independently associated with correct answers in multivariate analysis were the MS-expert subspeciality ($p<0.001$), young clinical practitioners ($p=0.02$), and the use of a computer instead of a smartphone to perform WML analysis ($p=0.03$).

Conclusion: Our results highlight the difficulties regarding WML analysis in clinical practice and suggest that radiologists and neurologists should rely on each other to ensure MS and related disorders diagnoses to limit misdiagnoses.

POSTER COMMUNICATIONS

2nd year PhD Students

P-30

Dev bio / Marine bio **DESHURAUD Romane** IRCAN

The role of telomeres in stress resistance of reef-building corals

Telomeres are nucleoprotein structures protecting the end of chromosomes. Their incomplete replication causes the size of telomeres (TLs) to decrease with each cycle of cell division, limiting cell renewal. They therefore contribute to both normal and pathological aging. Moreover, their structure is sensitive to many environmental stresses (e.g. temperature). The analysis of telomere length (TL) seems to be a promising tool to understand the consequences of climate change on living organisms. Currently, corals, which are sessile, ectothermic organisms with extreme variability in longevity, are among the organisms most affected by climate change. They thus constitute an interesting model to question the role telomeres play in the longevity of organisms in relation to the environment. During the TARA-Pacific expedition, the scientific community was interested in two genera of scleractinian corals and the results obtained allowed us to establish first hypotheses. Thus, the genus Pocillopora which is a shortlived and fast-growing coral seems to have their TL dependent on the environment while the genus Porites which is a long-lived and slow-growing coral seems to have their TL independent of the environment. New questions arise : what is the long-term relationship between temperature changes and TL and how can it be different for organisms living in the same environment. For this purpose, a longitudinal monitoring under natural conditions was set up in Moorea. Following the same sampling model realized during the TARA-Pacific expedition, three sites and three species were targeted with 10 colonies sampled per species per site. The first sampling corresponds to the TARA-Pacific expedition in 2016 and the last one in November 2022. In parallel, experiments in controlled environment and conditions have been set up in collaboration with the CSM and IRCAN: a temperature stress is applied on different species of corals: Porites and Pocillopora (the same two species of corals studied in natural conditions), Stylophora pistillata and Acropora sp. (branched corals similar to Pocillopora). They were placed in different aquariums at different temperatures. From the samplings and experiments conducted in the laboratory, gDNA extractions and then Southern blots are performed to analyze the TL. Our first results seem to show that telomeres are promising biomarkers and that they could help us to better monitor environment impact on organisms' physiology

POSTER COMMUNICATIONS

2nd year PhD Students

P-31

Immuno | **ZAIR Fairouz** | LP2M

Implication of the metabolism in dendritic cells activation during Psoriasis

Dendritic cells (DCs) are crucial players in the initiation of adaptive immune response. A key feature of DCs is their high capacity to capture antigens in peripheral tissues and migrate to draining lymph nodes (LN). In psoriasis, a chronic, systemic inflammatory disorder with multiple comorbidities, migratory IL23-secreting DCs drive T cell activation in LNs and IL-17 release. Metabolism controls key DC functions, but the metabolic pathways governing the activation, migratory processes and IL-23 secretion remain underexplored. My research project aims to decipher how metabolic components affect DC activation, migration and IL-23 production, trigger psoriasis and contribute to its exacerbation in metabolic disorders. Through the use of pre-clinical genetic models, pharmacological inhibitors and human samples, I will define the metabolic demands of IL-23 producing DCs. Furthermore, I will analyze the metabolic rewiring of DCs during psoriasis exacerbation by High Fat Diet and investigate the role of glucose metabolism in DCs migration in human psoriasis.

Keywords: Metabolism, Dendritic Cells, Psoriasis

POSTER COMMUNICATIONS

2nd year PhD Students

P-32

33 *Neurobiology* **CŒUR Estelle** CoBTeK

Improving PTSD diagnosis & treatment for patients with neurocognitive disorders

Key-words: PTSD; Neurocognitive disorder; diagnosis; music-therapy

Background: Posttraumatic Stress Disorder (PTSD) and Neurocognitive disorders (NCD) are worldly spread pathologies. Even though their origins are not related, they engender similar symptoms (cognitive and behavioral disorders) and brain changes (atrophy, plasticity). PTSD diagnosis becomes more complicated when patients also suffer from NCD. On top of that, treating PTSD for this population can be difficult because of cognitive impairment. The goals of this thesis are to improve (1) PTSD diagnosis and (2) treatment for patients with NCD.

Method: For the first objective (1), a new screening tool for PTSD used with patients with NCD was created and tested following the recommended guidelines. Regarding the second objective (2), the impact of a non-pharmacological approach on PTSD will be studied through a single case experimental design (SCED) with 5 patients suffering from NCD.

Results: Firstly (1), the new screening tool showed good global reliability (Cronbach's alpha = 0.75) and 13 domains were kept. As for the second objective (2), using music-therapy in the SCED engendered a decrease of the patients' PTSD until PTSD couldn't be diagnosed anymore. Results appeared stable in the 6 months follow-up.

Discussion: For the first goal, the new screening tool allows frequency and severity self-assessment of PTSD. The next step is to analyze its psychometric properties. Regarding the second goal, music-therapy appeared efficient for PTSD and its impact should be explored in detail. Thus, the next study will aim to replicate these results to generalize them.

POSTER COMMUNICATIONS

2nd year PhD Students

P-33

Physio - Electrophy / Neuro **GILBERT Nicolas** IPMC

Exploring the role of THIK potassium channels in nociceptive pathway

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Potassium channels play a crucial role in the nervous system, as they can affect resting membrane potential and modulate action potentials making them important targets for the search for new neuronal modulators. The K2P group of potassium channels are involved in various physiological functions mostly cardiac and neuronal. Recently, several K2P channels have been linked to the regulation of pain and mutation in K2P channels are associated with migraine and neurodevelopmental disorders.K2P channels are known to finely regulate neuronal excitability by hyperpolarizing their membrane.

Members of the Tandem pore-domain Halothane-Inhibited K+ channels subfamily (THIK1 and THIK2) are highly expressed in the Central and Peripheral Nervous System (CNS and PNS), but their role in the control of pain sensation has not been studied yet. Using in-situ hybridization technique (RNAscope), we have recently shown that THIK channels are co-expressed by non-peptidergic nociceptive neurons that express the Purinergic Receptor 2X3 (P2RX3) in PNS. These unmyelinated neurons are known to be involved in the transmission of slow nociceptive messages in Dorsal Root Ganglia (DRG) such as inflammatory and chronic ones. Moreover, RNAseq data shows that THIK1 and THIK2 are the most highly expressed K2P channels in microglial cells of the CNS, in which THIK1 has been linked to inflammasome activation. This suggests that these channels might play a role in inflammatory pain.

We are now investigating their role in transmitting sensory and nociceptive messages and whether these channels function as homomers or heteromers. Initial studies have shown that THIK2 knockout mice exhibit thermal allodynia, we are now investigating whether these mice exhibit mechanical or thermal inflammatory hyperalgesia. We aim to further explore the functions of THIK1 and THIK2 in nociception and differentiate the roles of homomeric and heteromeric forms of the THIK channels. This distinction is crucial for the development of specific pharmacology and targeted therapy.

POSTER COMMUNICATIONS

2nd year PhD Students

P-34

System biology | **LIN Peipei** | IRCAN

Deciphering the role of telomere-mitochondria connections during aging

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Telomere attrition and mitochondrial dysfunction are considered to contribute to the aging process. Telomeres are the structures located at the chromosome ends and are protected by a protein complex composed of six proteins (TRF1, TRF2, RAP1, TPP1, TIN2 and POT1) called shelterin. Recent studies have suggested that some shelterin subunits regulate mitochondrial functions independently of their role at telomeres, perhaps by their direct localization at mitochondria. However, whether telomere-mitochondria cross-talks play a role during aging are not known. To address this question, we re-visited the functions of all shelterin subunits at mitochondria using mouse embryonic fibroblast cells. The aim of this in vitro study is to find separation-of-function mutants having mitochondrial functions without damaging telomeres. For that, we knock downed the expression of all shelterin subunits and analyzed mitochondrial network using a fluorescent dye (MitoTracker). In addition, we used a mitochondria DNA (mtDNA) replication assay to study the potential role of shelterin in controlling mtDNA replication. The results showed that TRF2 impaired the mitochondrial network branches and mtDNA replication, while another shelterin subunit, TPP1, had the opposite effects. We went deeper in the characterization of the different domains of TRF2 and found that the N-terminal domain of TRF2 (B domain) was required for mtDNA replication, which can be uncoupled from its telomere protective property. To further investigate its role during aging in vivo, we will establish a knockin mouse model by CRISPR/Cas9-mediated modifications to delete the B domain of TRF2. Our study will hold the potential to drive future research on the roles of telomere-mitochondria cross-talks in aging.

POSTER COMMUNICATIONS

2nd year PhD Students

P-35

Cancer / Immuno | **KERRENEUR Emeline** | C3M

Reprogramming anti-inflammatory macrophages through cathepsin B and caspases inhibition

MDMs (monocyte-derived macrophages) are widely distributed innate immune cells that play an indispensable role in a variety of physiological processes, including organ development, host defense and anti-tumoral immunity. However, anti-inflammatory MDMs are also known to be deleterious in both solid and hematological cancers. Indeed, Tumor-Associated Macrophages (TAMs) or Leukemia-Associated Macrophages (LAMs) inhibit anti-tumoral responses and T cell-mediated cytotoxicity within the tumoral microenvironment, supporting tumoral growth, metastasis, and resistance to treatments. Therefore, understanding the mechanisms of MDMs generation/polarization might provide an opportunity for the design of novel therapeutic strategies to increase the effectiveness of cancer therapy. In this context, our team has developed a model to generate and polarize macrophages ex vivo. From peripheral blood of healthy donors, we purify human primary monocytes and differentiate them into immature macrophages (M0) upon CSF-1. Those macrophages are further polarized into a pro-inflammatory phenotype (M1) with LPS + IFN-γ, or into an anti-inflammatory phenotype (M2) with IL-4. With this model, we highlighted an original and highly specific mechanism of caspase activation involved in CSF-1-mediated macrophage differentiation, giving them non-apoptotic functions. Indeed, we showed a differentiation-specific mode of caspase-8 (CASP8) activation in CSF-1-treated monocytes that requires prior cathepsin B (CTSB) activation. This original activation of CASP8 leads to non-canonical cleavage and activation of CASP3 and 7, which in turn cleave several cellular proteins at sites different from those cleaved during apoptotic conditions. We took advantage of these specific cleavage sites to develop fluorescent synthetic substrates and new peptide inhibitors that allow us to measure and to abrogate non-apoptotic caspases activated in MDMs. Using pharmacological and siRNA approaches, we proved that CTSB and non-apoptotic caspases are also required for IL-4-induced immunosuppressive macrophage polarization contrary to pro-inflammatory macrophage polarization. Finally, we demonstrated that caspase inhibition can reprogram ex vivo anti-inflammatory macrophages towards a pro-inflammatory state. Altogether, our results highlighted the interest of targeting cathepsin B and non-apoptotic caspases to modulate the functions of anti-inflammatory MDMs, thus offering new therapeutic strategies in diseases, in which these pro-tumoral MDMs contribute to pathogenesis. Ex vivo and in vivo studies are in progress to 1) evaluate the activation of cathepsin B and caspases in TAMs / LAMs and 2) target them in appropriate mouse models of cancer (especially Acute Myeloid Leukemia, kidney, and breast cancer).

POSTER COMMUNICATIONS

2nd year PhD Students

P-36

Neurobiology **SOLYGA Mathilde** iBV

Regulation and function of neuronal ribonucleoprotein (RNP) complexes upon aging in Drosophila Melanogaster

Aging is a multifactorial process responsible for a general decline of physiological functions affecting organs at different rates. The brain is particularly impacted since the loss of cognitive, mobility and sensorial functions appear past 40 in humans. At the molecular scale, a number of primary hallmarks of aging (telomere attrition, epigenetic alterations, genomic instability, loss of proteostasis) induce a change in gene expression. Recent work, however, has suggested that the majority of age-dependent changes in gene expression in the brain may arise from changes in RNA translation profiles. How this is regulated is to date unclear. Work from our lab showed that ribonucleoprotein (RNP) granules, which are membraneless organelles composed of RNAs and RNA binding proteins (RBPs) involved in RNA spatio-temporal regulation, remodel upon in vivo aging in the Drosophila brain. This process is characterized by the coalescence of conserved RBPs such as Imp/ZBP1 and the DEAD-box RNA helicase Me31B/DDX6 in large, yet soluble, condensates. Remarkably, RNP granule remodeling associates with an increased recruitment of RNAs to granules and with their translational repression. Furthermore, both RNP granule remodeling and translational repression depend on the activity of the conserved kinase PKA. To date, how neuronal RNP granules are regulated by normal aging in a physiological context and their role in this process is still elusive. My project aims at identifying the molecular mechanism contributing to age-dependent regulation of RNP granules, through both unbiased and candidate approaches. I have so far identified Imp/ZBP1 as a target of PKA and mutated its potential phosphorylation site in-vivo. In parallel, I have studied others CRISPR point mutants affecting the catalytic domains of Me31B/DDX6. After confirming that these mutations specifically disrupt age-dependent RNP granules remodeling, I will investigate their consequences on aging physiology. This work aims to reveal for the first time the role of RNP granule during in vivo aging, and will open new perspectives related to the mechanisms underlying physiological aging.

POSTER COMMUNICATIONS

2nd year PhD Students

P-37

Structural bio **OUERTANI Sarah Amira** iBV

Deciphering the Molecular Grammar of Condensate Assembly

Gene expression is coordinated to cellular activity and adapted to stress. Transcription control can regulate whether an mRNA is transcribed, but it has become evident that rapid, efficient and adaptable responses to cues is achieved by controlling gene expression at the post-transcriptional level. A fundamental problem in cell biology is how the transcriptome organizes within the densely packed cellular space to allow efficient spatiotemporal regulation of mRNA expression?

Evidence suggests that post-transcriptional regulation in developing C. *elegans* oocytes is achieved by RNA binding proteins (RBPs) that dynamically regulate maternal mRNAs. Recent data from our team further suggests that translationnally repressed mRNAs can be found in a single molecule soluble form that can self-assemble into homotopic clusters that can further coalesce into larger multiphase heterotypic assemblies. These membrane-less mRNA super-assemblies are liquid-like bodies in which mRNAs and proteins are, for most condensates, the major components. These Biomolecular condensates can compartmentalize, concentrate and dynamically partition components, providing the cell with regulatory capabilities beyond canonical molecular regulatory mechanisms.

However, the mechanism of assembly and precise function of those different condensates remains an open question. Here, I propose to (1) dissect the mRNA sequence that drive condensate assembly, and to (2) test the consequence of RNA condensation on RNA:protein interactions. Finally, (3) I will address whether RNA condensation is correlated to translation and decay interplay. Using high resolution imaging approaches of mRNA localization within C. elegans gonads, I uncovered that mRNA 3'UTR sequences are unexpectedly insufficient to insure mRNA segregation into homotypic clusters. In addition, I adapted the OOPS biochemical approach, whose preliminary results suggest that preventing mRNA aggregation into granule disrupts RNA:protein interaction stoichiometries on a proteome-wide scale. Regarding the last question, I took advantage of a single molecule sensitivity RNA imaging approach to demonstrate that repressed/condensed mRNAs are protected from decay as compared actively translated single mRNAs. My preliminary results support models in which (1) mRNA condensation controls RNA:protein interaction stoichiometries within the cell, (2) mRNA condensation is associated with the protection of mRNA from decay.

POSTER COMMUNICATIONS

2nd year PhD Students

P-38

Neurobiology **CHATO ASTRAIN Isabel** IPMC

Targeting SUMOylation to rescue phenotypical alterations caused by the recurrent FMRP-R138Q missense Fragile X mutation

SUMOylation is a post-translational modification that can be triggered by the activation of the type I metabotropic glutamate receptors mGlu5R. It consists on the covalent but reversible binding of the SUMO polypeptide to specific lysine residues of the target proteins, including the Fragile X Messenger Ribonucleoprotein (FMRP). This protein is silenced due to the expansion of CGG repeats in the 5'UTR, leading to the most common monogenic cause of intellectual disability called Fragile X syndrome (FXS). FXS is thus a rare genetic disease characterized by a range of developmental manifestations including learning disability and cognitive impairment. Several FXS causing mutations have been also described, such as the missense mutation R138Q in *FMR1* gene, located 8 residues away from the active K130 SUMOylation site of FMRP. In the laboratory we engineered a knock-in mouse model *Fmr1*-R138Q carrying this FXS mutation. The lab demonstrated that this R138Q mutation does not affect the ability of FMRP to bind and transport specific target mRNAs, despite the apparent dysfunction on the mutated protein. We hypothesize that the activity-dependent SUMOylation of the R138Q FMRP mutant is impaired. The aim of my project is to use SUMO-targeting drugs in FMRP-R138Q-KI neurons to rescue the altered spine density and AMPAR surface expression caused by this FXS mutation.

POSTER COMMUNICATIONS

2nd year PhD Students

P-39

Biochemistry **SALAME Sarah** IPMC

Understanding the role of sn-1 acyl chains of membrane lipids

Sarah Salame, Lucile Fleuriot, Delphine Debayle, Takeshi Harayama

Biological membranes are composed of diverse lipids that can have >1000 distinct chemical structures, which is largely contributed by the multiple pairs of esterified fatty acids (with varying chain lengths and unsaturations). Lipid fatty acid composition affects various biological function through its effects on membrane physical properties and membrane-associated protein functions. Fatty acids are found in two different positions in glycerophospholipids, at the *sn*-1 and *sn*-2 position of the glycerol backbone, and are transferred into lipids by diverse enzymes called acyltransferases that differ in substrate preferences. They incorporate fatty acids during both de novo synthesis of glycerophospholipids and their remodeling pathway, in which fatty acids are exchanged. In contrast to the presence of multiple studies showing the roles of various *sn*-2 fatty acids, sn-1 fatty acids, mainly being saturated or monounsaturated C16 or C18 fatty acids, gained little attention. It remains unclear how acyltransferases regulate fatty acids at this position. Recent research suggested that fatty acid at the *sn*-1 position also have important biological roles. One example is the lateral organization of plasma membrane proteins by phosphatidylserine, which was attributed to species having stearic acid (C18:0) at the *sn*-1 position. Therefore, it is crucial to understand better how *sn*-1 fatty acids are differently incorporated, and which functions they have. Here, I will present my thesis project that attempts to study the metabolic regulation and biological functions of *sn*-1 fatty acids, especially the role of phosphatidylserine with *sn*-1 stearic acid, in the signaling downstream of glycosylphosphatidylinositol-anchored proteins (GPI-APs). To investigate comprehensively how acyltransferases regulate *sn*-1 fatty acids, we generated a library of HeLa mutant lacking acyltransferases (in total 21). We analyzed some of the mutant cells by lipidomics and we found some enzymes responsible to incorporate stearic acid at the *sn*-1 position of phosphatidylserine. The lipidomics dataset also revealed other novel functions of distinct acyltransferases. In the future, combinatorial mutants of distinct acyltransferases will be generated to further manipulate sn-1 fatty acids and study their contribution on GPI-AP signaling.

POSTER COMMUNICATIONS

2nd year PhD Students

P-40

Marine bio **ANDREONI Rita** IRCAN

Apoptosis is a regeneration-specific process for Nematostella vectensis.

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Regeneration is the ability to repair tissues, organs, or even whole bodies afterinjury. To gain novel insight into the molecular and cellular mechanisms underlying whole-body regeneration (WBR), a recent study compared embryonic development and regeneration in the sea anemone, *Nematostella vectensis,* suggesting that apoptosis is a regeneration-specific process required to initiate regeneration via a tissue crosstalk. However, the precise roles and their chronology during regeneration in *Nematostella* are yet to be determined.

We performed a detailed characterization of the tissular crosstalk responsible for the initiation of regeneration and in silico identification of pro and anti-apoptotic genes was performed as well as their spatio-temporal expression analyzed. To gain functional insight into the role of apoptosis during embryonic development and regeneration, we used the pharmacological pan-caspaseinhibitor Z-VAD and assessed the resulting phenotype using tissular, molecular and cellular markers.

This work revealed the precise tissular dynamics during the wound-healing process that leads to the tissue crosstalk at the origin of the regeneration process. Inhibition of apoptosis not only prevents this crucial tissue crosstalk, but also, all subsequent events such as cell proliferation and the reformation of lost body parts. Our data further suggest that apoptosis and MEK/ERK signaling are both activated shortly after injury and act in parallel to coordinate the regenerative response.

This work highlights the regeneration-specific role of apoptosis in *Nematostella* and paves the way to a detailed analysis of the molecular signals, including the apoptosis-dependent ones, underlying the regeneration-inducing tissue crosstalk.

POSTER COMMUNICATIONS

2nd year PhD Students

P-41

Immuno **MOSKALEVSKA Iryna** IRCAN

Role of senescence immune checkpoints in immunosurveillance and their implications in agerelated diseases

Aging is characterized by a gradual decline of physiological functions causing age-related diseases and increasing vulnerability against cancer. At a cellular level, aging is associated with a progressive accumulation of senescent cells. Senescent cells are involved in tissue homeostasis on one hand but, on another hand, their accumulation can lead to pathological aging processes. The events regulating the elimination of senescent cells by the immune system are still poorly known. Understanding how they accumulate in tissues would allow simultaneously to better understand the etiology of age-related diseases and to improve the design of new therapeutic strategies against age-related diseases and cancer. We suggest that senescent cells developed specific immune checkpoints (Senescence Immune Checkpoints or SIC) to escape the immune system by progressively increasing immunosuppression, altering the immune composition of the senescence micro-environment, accelerating the aging process. By analogy with cancer immunoediting, our hypothesis is that during aging, this accumulation induces an equilibrium where the senescent and immune cells apply a mutual adaptation force favoring a coevolution. The aim of our project is to understand the consequences of the interactions between senescent and immune cells on longevity and development of age-related diseases to potentially reveal a “senescence immunoediting” by quantitative (RNA seq) and qualitative (flow cytometry, in vivo experiments) approaches. Keywords : senescence, immune checkpoints, Natural Killer

POSTER COMMUNICATIONS

2nd year PhD Students

P-42

Dev / Neuro **DALLORTO Eleonora** iBV

Nr2f1 haploinsufficiency leads to reduced inhibition of hippocampal DG neurons and mitochondrial dysfunction in the adult mouse brain

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The Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS; OMIN#615722), is a rare neurodevelopmental disorder caused by mutations in the NR2F1 gene, also known as COUP-TFI, a transcriptional regulator playing pleiotropic functions in brain development. Most variants in the NR2F1 gene of patients described so far are deletions and/or mutations leading to haploinsufficiency or dominant negative effects. Although BBSOAS is characterised by a wide array of clinical features, intellectual disability, visual impairment, and autistic traits are the most common. In this study, we analysed the effects of Nr2f1 haploinsufficiency in the postnatal brain of a constitutive Nr2f1 heterozygous mouse model (i.e., *Nr2f1-HET*), which is known to recapitulate some of BBSOAS neurological phenotypes, among which optic nerve atrophy and altered learning/memory.

By exploiting the adult hippocampal dentate gyrus (DG) - a site where Nr2f1 is highly expressed and with key roles in cognitive processes such as learning and memory - our *in situ* analyses revealed (i) morphological alterations in adult-born immature neurons, (ii) increased expression of immediate early genes (e.g., Npas4, c-fos) and (iii) reduced mIPSCs frequency and inhibitory synapses in the DG mature granule neurons of *Nr2f1-HET* mice. These results suggest a possible excitatory/inhibitory imbalance that could impact on the maturation of adult-born neurons and likely influence cognitive functions. Future investigations are needed to dissect the underlying mechanisms and in particular the cell-intrinsic versus cell-extrinsic components of the observed phenotypes. Therefore, we started to investigate a possible involvement of Nr2f1 haploinsufficiency in mitochondrial dysfunction. First, by *in silico* analyses we showed that among the direct Nr2f1 putative genomic targets many are downregulated in *Nr2f1-HET* mice, of which about the 8% encoding for mitochondrial proteins. Moreover, by Western Blot assay we quantified the levels of selected key mitochondrial proteins in isolated mitochondria from the whole brain and found reduced levels of proteins needed for mitochondrial fusion and mitochondria-related metabolism and respiration in *Nr2f1-HET* mice compared to controls. Considering that multiple clinical features of BBSOAS patients are compatible with an altered mitochondrial function in the nervous system, our data strongly support a direct involvement of mitochondrial dysfunction in BBSOAS pathogenesis.

POSTER COMMUNICATIONS

2nd year PhD Students

P-43

Plant bio / Microbio **NAZARET Fanny** ISA

RoxR, a redox-sensing regulator of Sinorhizobium meliloti, is crucial for symbiotic infection of Medicago truncatula roots.

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Sinorhizobium meliloti is a soil bacterium that can establish nitrogen-fixing symbiosis with the legume *Medicago truncatula*. This interaction is initiated in the rhizosphere by a molecular dialog between the two partners. Subsequent plant root infection leads to the formation of a novel root organ, the nodule, where bacteria differentiate into bacteroids reducing the atmospheric nitrogen into ammonium. The regulation of *S. meliloti* intracellular redox state plays a major role in nodule development and functioning. Indeed, bacteria are exposed to reactive oxygen species (ROS) produced by the plant at different steps of the interaction, and several mutants of the antioxidant defense are affected in nodule development. Moreover, ROS are known to play a role in redox signalling in particular by modifying the activity of redox-sensing transcriptional regulators (TRs). In bacteria, many of them belong to the MarR family. This work focused on a *S. meliloti* MarR-like TR, called RoxR, expressed during symbiosis. Using both *in vitro* and *in vivo* approaches, we demonstrated that RoxR is a repressor that binds to *its own* promoter, and is inactivated by some oxidants (NaOCl, peroxides). The deletion of *roxR* did not significantly affects the growth rate of *S. meliloti* under normal conditions. However, using a redox-sensitive GFP, we showed that the mutant has an increased capacity to maintain intracellular redox homeostasis upon oxidant treatment. *In planta*, the deletion mutant was strongly affected in nodulation and nitrogen fixing capacities. An analysis of the expression of *M. truncatula* marker genes showed that the *ΔroxR* mutant induced an altered plant response at early infection stages. In addition, microscopic analyses of nodules showed that the *ΔroxR* mutant induced the formation of many non-invaded pseudonodules and rare nodules. Altogether, these results suggested that RoxR plays a key role in the redox regulation of symbiosis.

References

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POSTER COMMUNICATIONS

2nd year PhD Students

P-44

Physio - Electrophy **KAYATEKIN Mete** LP2M

Activation of the LRRC8/VRAC anion channel in macrophages plays a central role in micro-crystallin joint inflammation

Mete Kayatekin1 , Twinu Wilson Chirayath2,4, Matthias Ollivier3 , Isabelle Rubera1 , Laetitia Clotaire1 , Hélene Hirbec3 , Francois Rassendren3 , Vincent Compan3 , Korng Ea2,4, Christophe Duranton1

Monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals depositions are responsible for interleukin (IL-1β) mediated human inflammatory joint diseases (gout and pseudogout, respectively). In macrophages, the release of IL-1β depends on NLRP3 inflammasome activation following exposition to danger associated molecular patterns (DAMPs) such as crystals, nanoparticles, ATP, …. Beyond the variety of stimuli, a decrease of osmolarity is also known to induce NLRP3 inflammasome activation leading to IL-1β secretion. To fight against uncontrolled volume change, cells have developed a compensatory mechanism named regulatory volume decrease (RVD). RVD depends on a ubiquitously expressed channel called Volume Regulated Anion Channel (VRAC), which allows the cell to return to its initial volume through the loss of chloride ions that drives water efflux. This LRRC8/VRAC channel is composed by the mandatory subunit LRRC8A and at least one other member of the LRRC8 family (5 members, A to E) to create hetero-hexameric functional VRAC channels. In this work, we investigated the link between the LRRC8/VRAC channels and the crystals-mediated NLRP3 inflammasome activation. Elisa experiments performed on WT THP-1 cells (human monocytic cell line primed with 500nM PMA) showed that sterile MSU or CPP crystals exposure (200µg/ml, 3h) triggered an IL-1β release. This IL-1β release was reduced by blocking VRAC channels with DCPIB (a selective VRAC inhibitor, 20µM) or by silencing the mandatory LRRC8A subunit (LRRC8A KD). We next investigated cell volume change under crystals exposure over a 2h period by using calcein fluorescence quenching technique. WT THP-1 cells treated with DCPIB or LRRC8A KD THP-1 cells showed an absence of RVD mechanism after crystal exposure. Since LRRC8/VRAC is permeable to a vast set of molecules and that ATP is an inflammasome activator, we investigated whether crystal-induced NLRP3 inflammasome activation is controlled by LRRC8/VRAC -dependent ATP permeability that drives purinergic signalling pathways. We first showed, by using patch-clamp technique, that LRRC8/VRAC exhibited an ATP conductance under a hypoosmotic challenge that is blunted by DCPIB or in LRRC8A KD cells. Then, by using luminescent experiments, we showed that a 2h incubation with crystals induced an increase of extracellular ATP concentration. Similar experiments performed in the presence of DCPIB or by using LRRC8A KD cells showed a significant reduction of crystals-mediated ATP release. In conclusion, these results suggest that LRRC8/VRAC channel plays a crucial role in the crystal mediated NLRP3 inflammasome activation. Further in vivo experiments must be done to decipher the exact contribution of LRRC8/VRAC activation during micro-crystals-mediated joint inflammation.

POSTER COMMUNICATIONS

2nd year PhD Students

P-45

Neurobiology **BADOT Céline** IPMC

Role of C99 oligomerization and exosomal spread in Alzheimer's disease

Up to recently, the amyloid β (Aβ) peptide was thought to be the trigger of AD, but the consistent failure of therapies aiming at decreasing Aβ production or neutralizing it once formed have questioned this dogma and have suggested a pathogenic role of alternative candidates. Indeed, such a hypothesis was validated by the fact that the accumulation of the direct precursor of Aβ, C99, was found to cause early Aβ-independent endolysosomal dysfunction and consequently preventing the clearance of neurotoxic molecules. Recently, our lab showed that exosomes, small endosomal-originating extracellular vesicles, purified from AD models contain C99 existing as both monomers and oligomers, but the role of C99 oligomerization remains unknown. Since exosomal secretion is known to increase when lysosomal/autophagic degradation is compromised but also participate to the prion-like transmission of neurotoxic proteins, we hypothesize that particularly the spread of toxic C99 oligomers could contribute to AD pathogenesis. Thus, my aim is to characterize the intracellular neo-oligomerization of C99 and the exosomal spread of toxic C99 oligomers using bimolecular fluorescence complementation (BiFC). This method is a protein interaction assay based on the principle that a fluorescent protein (Venus) is reassembled from its two complementary non-fluorescent fragments (N-terminus and C-terminus) when an interaction occurs between two proteins (here two molecules of C99 tagged with VN and VC) thus giving rise to a yellow fluorescence. After validation of the optimal protocol for biochemical and imaging analysis in transiently transfected HeLa cells, this new tool allowed us to specifically delineate the trans-Golgi localization of neo-oligomerization and trafficking of oligomeric C99 to the endolysosomal system by high-resolution microscopy. These results support the feasibility of tracking the exosomal spread of C99 fluorescent oligomers. Interestingly, biochemical analysis revealed that oligomerization decreases the cleavage of C99 by γ-secretase supporting an accumulation and a potentiation of Aβ-independent toxicity of oligomeric C99. Hopefully, the outcomes of this project will be a step forward in understanding the mechanisms leading to AD pathogenesis, which could ultimately provide new windows for therapeutic intervention.

POSTER COMMUNICATIONS

2nd year PhD Students

P-46

Immuno **BELDI Ghada** LP2M

Title: Modulation of the TH17 lymphocytes immune response using anti-TNF biotherapies in chronic inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic condition that is characterized by severe gut inflammation and diverse extra-intestinal manifestations, the common one being bone destruction. This bone loss is due to the overproduction of inflammatory mediators (TNFa, RANKL, IL17) by activated immune cells that increase the differentiation of bone-resorbing osteoclasts.

Th17 lymphocytes massively infiltrate the inflamed intestine of IBD patients, where they produce IL-17 and other “pathogenic” cytokines, amplifying the inflammatory process. These IL17 secreting lymphocytes are doted with high plasticity and were proven to be able to shift into a TH1 like phenotype by producing the TH1 signature cytokine IFNg.

Using the model of IBD induced in RAG $1^{-/-}$ mice by transfer of naïve CD4 T lymphocytes, we have demonstrated the existence of TH17 lymphocytes positive for TNFa and RANKL, a population that promotes osteoclastogenesis and thus the associated bone loss. These data highlight the involvement of the TH17 lymphocytes in the extraintestinal manifestations of Inflammatory bowel disease.

Anti-TNF therapy remains the first line of treatment for IBD, it overcomes gut inflammation and bone destruction. In the RAG $1^{-/-}$ IBD mouse model, we showed that treating the mice with anti-TNF reduced the gut inflammation and the associated bone loss and this is due to the reduction of the TH1 like TH17 lymphocytes and the decrease of the RANKL levels and osteoclasts differentiation. Despite the improvement of intestinal lesions and bone status psoriasis like skin lesions appeared.

Deciphering the molecular/cellular dialog between the gut, bone marrow and the effect of the anti-TNF treatment on the TH17 cell population remains therefore a major goal.

Keywords: IBD, TH17, anti-TNF

POSTER COMMUNICATIONS

2nd year PhD Students

P-47

Physio - Electrophy / Plant bio **RANTY-ROBY Sarah** ISA

Identifying molecular plant functions targeted by root knot nematode nuclear effectors

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Root knot nematodes (RKN) are endoparasitic worms that invade plant roots causing economically important damages worldwide as they have a wide host range. Furthermore, RKN populations are predicted to geographically spread and to increase with climate warming. Through their stylet (a syringe-like organ), these pests inject proteins into plant cells to manipulate diverse functions to their advantage and to escape plant defenses. Indeed, RKN induce in the root, the formation of giant polynucleate cells, which constitute their feeding sites to drain plant nutrients thus affecting plant yield. Understanding the molecular dialog between plant and root knot nematodes is therefore of high interest to build new strategies of plant protection against these parasites.

Transcriptomic analysis of the interaction between tomato and Meloidogyne incognita was performed in order to identify RKN putative secreted proteins, named putative effectors, more strongly expressed in planta. For these predicted effectors, we performed in situ hybridization in order to see whether they were expressed in the RKN salivary secreting glands suggesting effector injection in planta. Then, in planta localization of these candidates proteins by agro-infiltrating GFP-tagged effectors in tobacco was performed to select RKN effectors targeting the plant cell nucleus. Finally, yeast two-hybrid screening was also undertaken in order to identify potential targets of these effectors in the tomato plant. Results will be presented for such putative effectors and discussed in the context of the biology of the interaction.

POSTER COMMUNICATIONS

2nd year PhD Students

P-48

Developmental bio / Neuro **PIOVANI Paolo** iBV

Modelling BBSOAS, a neurodevelopmental syndrome, in mouse models and human retinal organoids

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1.Université Côte d'Azur, CNRS, Inserm, iBV, Nice, France.

Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS) is a rare genetic disease caused by haploinsufficiency of NR2F1, a key transcriptional regulator that is highly expressed in the developing central nervous system. Patients with BBSOAS experience a range of symptoms including intellectual disability, developmental delay and visual system impairment, primarily due to optic nerve atrophy.
The only available BBSOAS mouse model to date (Nr2f1 null line) has proved a key role of Nr2f1 in regulating the number of retinal ganglion cells (RGCs) and, as a consequence, impacting optic nerve development and maturation. However, this mouse model has limitations as it only partially mimics BBSOAS symptoms and fails to replicate the heterogeneity of this syndrome. Additionally, the specific function of NR2F1 in human retinogenesis remains unknown as a human-like BBSOAS model is currently unavailable.

To investigate NR2F1 function in visual system development, my project employs a dual approach. On one hand, two new mouse lines with patient-specific NR2F1 gene alterations (a point mutation and a truncation) were investigated. These mutations cause a stronger phenotype resulting in delayed differentiation of retinal progenitors into RGCs, suggesting that patient-specific mutations can have a greater impact compared to the simple loss of one or two gene copies.

On the other hand, we set up an *in vitro* system to challenge NR2F1 function in human cells. In the past decade, the use of brain organoids derived from human induced pluripotent stem cells (hiPSCs) has emerged as a promising tool for modelling neurodevelopmental diseases at a cellular and molecular level. In this perspective, I optimized a 3D retinal organoid protocol for two different hiPSC lines and found that tight control of the initial number of cells forming hiPSC aggregates is crucial for the formation of neuroepithelia expressing *bona fide* retinal cell markers. Ongoing experiments are evaluating whether hiPSCs carrying mutated forms of NR2F1 show impaired rate of proliferation and delayed differentiation of retinal progenitor cells, as well as abnormal RGC viability, compared to their respective parental control lines.

Taken together, our double experimental approach utilizing *in vivo* and *in vitro* models revealed a key role for NR2F1 in controlling the ratio between retinal progenitor proliferation and RGC differentiation, ultimately affecting optic nerve formation in a mutation-specific manner.

POSTER COMMUNICATIONS

2nd year PhD Students

P-49

Cancer / Immuno **PIERANTONI Alessandra** IRCAN

A role for p16High oocytes in postnatal life.

Cellular senescence is a state of permanent cell cycle arrest in response to different stress factors. This leads to the activation of two different signaling pathways, p53/p21 or p16/pRb. Excessive accumulation of senescent cells in tissues can negatively affect them and create a proinflammatory microenvironment that is suitable for the onset and progression of various age-related diseases, including cancer.

This project is related to the analysis of senescent and senescent-like cells that express a high level of a cell cycle arrest gene p16 (Grosse et al., 2020, Cell Metabolism). Specifically, we found that there is a noticeable fraction of mouse oocytes that express this marker, this phenotype is produced only in 9% of the offspring. We followed up on mice born from these oocytes in adulthood and found several interesting phenotypes related to cancer and immune response.

In this respect, we believe that activation of p16High senescence program during oogenesis sets up particular epigenetic changes that are partially maintained in the adult state. This activation is not dependent on age or sex and may be a result of the activation of senescence or a senescence-like program induced in response to strong DNA damage.

In turn, these epigenetic changes are responsible for phenotypes observed in adult mice. In this respect, during adulthood, these mice show a drop in the percentage of immune cell subsets, leading to an exhaustion of the immune system compared to wild-type mice. In tumorigenic models, they are less sensitive to cancer treatment and characterized by the exhaustion of the immune system.

This project may provide insight into the link between epigenetic changes during early embryonic development and phenotypes in adulthood, which could also apply to humans.

POSTER COMMUNICATIONS

2nd year PhD Students

P-50

51 *Bio modeling* **WINTER Rémy** UR2CA

Development and standardization of a three-dimensional wrist model for surgical planning

Introduction: 3D preoperative planning is increasingly used with software already available for the shoulder, knee, and hip, but still lacking for the wrist. Although manually constructed planning models exist for forearm surgery, the measurements are user-dependent, and their time-consuming construction makes them unsuitable for clinical practice. The aim of this study is to propose an automatic 3D measurement model at the distal radius which measurements agree with current manually constructed models using point landmarking.

Methods: A database of 40 whole forearm CT scans was used for this study. On the one hand, the measurements in frontal plane (radial inclination) and in sagittal plane (palmar tilt) were obtained by standard manual landmarking of points. On the other hand, a 3D automatic segmentation and modelling algorithm was developed to obtain distal radius measurements from a series of DICOM images. These angles were calculated based on the principal axis of the geometric shape that best fit the radiocarpal surface. The agreement between the two measurement methods was then analyzed by the Bland-Altman method. The intra- and interoperator measurement variability was also analyzed for the manual method.

Results: Radial inclinations and palmar tilts obtained by the 3D model were on average 2° and 1.5° lower respectively than the hand-made point landmarking model. Ninety-five percent of the variability between the two measurement methods was between [-5°; 1°] for radial inclination and [-6°; 3°] for palmar tilt. For the manual method, the intra- and inter-operator variability of radial inclination was 1.7° and 1.8° respectively, and 3.2° and 4.7° for palmar tilt.

Discussion: This study shows an acceptable agreement between the measurements made by the 3D model and the manual methods. There is no consensus in the literature on the choice of points to be used for palmar tilt measurement, resulting in variations of more than 6° between methods. This highlights one of the advantages of the 3D model as measurements generated from the entire radiocarpal surface allow us to homogenize and improve our definitions of distal radius measurements. Furthermore, there is no variability of the results from the automatic modelling software in contrast to manual methods.

Conclusion: The 3D model developed is a satisfactory alternative to manual positioning methods. In addition, it contributes to improved reproducibility and considerable time savings.

POSTER COMMUNICATIONS

2nd year PhD Students

P-51

Biochemistry / Physio **LEGROUX Ilona** IPMC

Effect of alpha-Linolenic acid and its oxygenated derivatives linotrins on pancreatic beta-cell in type II diabetes

Ilona Legroux, Guillaume Daziano, Sophie Béraud-Dufour, Thierry Coppola, Patricia Lebrun and Nicolas Blondeau

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Type 2 diabetes (T2D) is a major public health problem, affecting 380 million people worldwide. This disease is defined by a chronic hyperglycemia and the main physiopathological characteristics are insulin resistance and insulinopenia. This insulin deficiency may be due to a deterioration of insulin secretion or a reduction of beta-pancreatic cells mass that secrete insulin. Therefore, preserving the mass of beta-cells and their function is an avenue for the treatment or prevention of T2D.

In this context, having a healthy and balanced diet could play a major role in resistance to T2D. Thus, we are interested in alpha-linolenic acid (ALA), an essential polyunsaturated omega-3 fatty acid found in plant, which is not synthesized by the body and must therefore be provided by food. ALA is known for its anti-inflammatory and protective properties, especially on cell death, and to improve insulin sensitivity. Consequently, we also are interested in its oxygenated derivatives that could potentially have similar effects. Indeed, the double oxygenation of ALA by 15-Lipoxygenase (15-LOX) produces oxylipins called "linotrins" that have strong anti-inflammatory properties. Interestingly, 15-LOX is a lipid-peroxidating enzyme implicated, *inter alia*, in the pathogenesis of inflammation and diabetes hence the interest to study and better understand the effects of ALA and linotrins on pancreatic beta-cell in T2D.

In this project, we focused on the insulin deficiency observed in T2D.

We tested two hypotheses that ALA and linotrins 1/ protect beta-cells against cell death and provide their survival, and 2/ potentiate insulin secretion by beta-cells in response to glucose. We demonstrate that ALA and *in vitro* synthetized linotrins "Suj34" and "Suj47" protect beta-cells against death induced by Interleukin-1beta and staurosporine. Using biochemical and cell biology techniques, we reveal that the protective effects of ALA, Suj34 and Suj47 rely on the phosphorylation and activation of the transcription factor CREB. Finally, we show that ALA increases glucose-stimulated insulin secretion *in vitro* and we are currently testing Suj34 and Suj47 to see if they have a similar effect. Subsequently, we would like to clarify the underlying mechanisms involved in the potentiation of insulin secretion by our molecules and test them on an *ex vivo* and *in vivo* model.

This project could then highlight ALA and its linotrin derivatives as innovative therapeutic tools against T2D.

POSTER COMMUNICATIONS

2nd year PhD Students

P-52

Immuno / Microbio **DURAND Tristan** other

Interactions between two major honey bee viruses throughout the lifespan of honey bees

Honeybees are commonly infected by various viruses (Beaurepaire et al., 2020). Viral replication is usually kept under control by honey bees at a relatively low level without showing clinical signs (covert infection). However, exposition to biotic (e.g. mites, microsporidia) or abiotic (e.g. pesticides, temperature) factors can alter this equilibrium, triggering pronounced viral replication and outbreaks of clinical signs (overt infection). It has been suggested that viral interactions can play a role in triggering such overt infections (Dubois et al., 2020 ; Remnant et al., 2019 ; Ryabov et al., 2016). However the nature of these interactions and their underlying mechanisms are currently understudied. The method of viral exposition, its chronology, the viral strains and the developmental stage of the infected bee are as many factors that can be involved in these mechanisms. In these series of experiments, we focused on two major honey bee viruses causing disease specifically in honey bee brood while replicating in adults as well : *Sacbrood virus* (SBV) can disrupt the development of honey bee larvae (Bailey et al., 1964; Wei et al., 2022), and the two master variants of deformed wing viruses (DWV-A and DWV-B) can alter wing development during the pupal stage, leading to lifespan reduction in adults and their inability to fly (deMiranda et al., 2010; Paxton et al., 2022). In a first experiment, we co-inoculated white-eyed honey bee pupae with different strains of SBV and DWV by injection. We measured their mortality and the appearance of clinical signs at emergence, then quantified the viral load in each pupae for these viruses. In a second experiment, we marked and co-inoculated emergent bees, either simultaneously or sequentially, either orally or by injection, before introducing them to a hive connected to an optical counter. Some of these bees were collected at two different intervals post-inoculation as to quantify viral loads while others were left in the hive as to measure their individual foraging behaviour using the optical counters. Our results show that SBV and DWV can interact synergistically or competitively depending on the inoculated virus strains and developmental stage of the bee. In addition to the consequences of these interactions on individual bee health, they may have implications on the evolution and spread of these two viruses. Future directions regarding the study of the mechanisms behind these interactions will also be discussed.

POSTER COMMUNICATIONS

2nd year PhD Students

P-53

System biology | **MIRA Thierry** | RETINES

Feasibility, acceptability and preliminary effects of advanced practice nurse (API) consultation activity for HIV pre- and post-exposure prophylaxis

Mira Thierry

Laboratory RETINES

Introduction

The HIV epidemic has been going on for more than 35 years... 35 million men, women and children have died of AIDS since the beginning... More than 37 million people live with it today... But more than 14 million people are not diagnosed... The tools exist to contain the epidemic... To increase the decline observed since 2017, it is necessary to expand the offer of care by opening up certain tools of combined prevention to advanced practice nurses. Combined prevention is available in several tools, including treatment as tools to prevent transmission, by treating from the positive diagnosis, by treating from the accident of exposure by treating in pre-exposure.

Materials and methods

Do pre- and post-exposure prophylaxis consultations to Viruses! It is the same acute or chronic accident that is sought to be prevented through longer, more holistic consultations, so as to allow these people to retain these people and to associate over the long term regular follow-up and screening for all sexually transmitted diseases, as well as prevention messages. The goal is to reach 90% of people diagnosed 90% of people treated and 90% of people who are undetectable and non-contagious. Design of the study: experimental, pilot and historico-propective.

Results and discussion

The expected results are a care offer more in line with this type of consultation, which revolves around the libido of the people who consult, and therefore personalized consultations focused on a community where prevention messages can more easily spread. Try to objectively visualize the changes induced in patients' behaviors by this nursing support, to limit their impact and ultimately change the recommendations on this subject.

Conclusion

All international experiences including nurses in the system at different levels are positive. Nursing interventions appear to improve patient adherence, most often induce prevention messages and take greater account of individual community and individual needs.

By multiplying and diversifying the supply of care, access for all is facilitated.

POSTER COMMUNICATIONS

2nd year PhD Students

P-54

Cancer | **KAHI Michel** | C3M

Inhibition of hypusination reprograms Prostate Cancer cell metabolism and decreases aggressiveness

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Prostate cancer (PCa) is a major public health problem, and the treatment of advanced stages remains not curable. Our team is interested in innovating therapeutic approaches targeting cancer cell metabolism. Cancer cells adapt their metabolism to resist various stresses and treatments and to provide the metabolites, energy and co-factors required for their proliferation and progression. Here, we focus on the polyamine/hypusination pathway which is associated with lower disease-free survival in PCa. Hypusination is a unique post-translational modification of the eukaryotic translation initiation factor 5A (EIF5A). This reaction is dependent on spermidine (a polyamine) and it is regulated by two enzymes, the deoxyhypusine synthetase (DHPS) and the deoxyhypusine hydroxylase (DOHH). Hypusination is involved in several cellular processes such as autophagy, metabolism, senescence, and differentiation, however, the mechanism by which it is implicated in tumor growth and metastasis is still unclear. To elucidate its role in PCa, we inhibited the enzymes that catalyze this reaction and investigated the effects on PCa cells aggressiveness and metabolism. We have shown that inhibition of hypusination decreases cell growth, cell migration and mitochondrial respiration, three biological processes implicated in PCa aggressiveness and metastasis. In addition, our metabolomic and proteomic analysis revealed significant changes in cancer cell metabolism. Our results highlight a potential therapeutic opportunity for PCa that target hypusination and could be used for clinical applications.

POSTER COMMUNICATIONS

2nd year PhD Students

P-55

Microbiology | **ORTIS Morgane** | Micoralis

Relationship between endurance sports and oral microbiota.

The oral cavity is home to the second largest and most diverse microbiome after the gut, including fungi, viruses, protozoa and over 700 species of bacteria. An oral microbiome in eubiose is crucial for both oral health as well as the systemic health, which has been confirmed for several pathologies, such as diabetes, cardiovascular, pulmonary, and neurodegenerative diseases... Thereby, oral biomarkers could be used to predict future alterations in oral and systemic health… However, only limited data describes the homeostasis in the oral cavity of healthy individuals, in particular, concerning the impact of their lifestyle (i.e. diet, environment, physical effort, stress, etc.).

The practice of regular physical activity is generally considered beneficial for maintaining a good health, but at high intensities, it can promote infections and the development of diseases. This is particularly observed in the oral cavity where many high-level athletes experience a deterioration of the oral health. Although the mechanisms of this deterioration are still unknown, these observations suggest a strong connection between oral biology and physical condition. Our hypothesis is that a sedentary lifestyle as well as high-intensity physical activity induces impoverishments and/or changes in the oral microbiota compared to a regular practice of reasoned physical activity.

This study will include 200 participants divided into 3 groups practicing different levels of endurance physical activity according to the criteria described by the World Health Organization (WHO) (i.e. insufficient, recommended, high). The oral biomarkers will be quantified from saliva samples, due to the many benefits of this sampling method compared to blood samples, e.g. minimizing stress, offering the possibility to test a large number of individuals in a bulk sample while being economically advantageous.

An inherent limitation of the 16S rRNA gene sequencing method is that it only provides the relative abundances of individual community members. Complementary approaches, such as qPCR, are then necessary to quantify the microbial communities. However, this method is expensive, labor-intensive, time-consuming, requires a large volume of reagents, and only tests a few targets at one time. To overcome this, we aim to develop an array specialized in the detection of oral microbiota, thanks to the Biomark™ HD high-throughput system from Fluidigm®, allowing us to obtain 4608 endpoints (96 samples × 48 targets) in a single qPCR run, making it more cost-effective and reducing reaction volume to 10nL scale. This array allows the quantification of 40 oral bacteria, 20 specific to the commensal oral microbiota and 20 specific to periodontal diseases, i.e. gingivitis and periodontitis. The plate also allows the detection and quantification of the 8 human herpes viruses that maintain oral microbial dysbiosis.

POSTER COMMUNICATIONS

2nd year PhD Students

P-56

Neuro / Structural bio **KALBFEIS DIT DARNAS Aurélien** iBV

Physiological importance of RBP modulatory: Imp protein study

Aurélien Darnas, Florence Besse

The spatiotemporal control of subcellular distribution of RNAs has recently emerged as a fundamental post-transcriptional mechanism involved in both physiological processes and the progression of various diseases such as cancer or neurodegenerative diseases. Tight and dynamic compartmentalization of RNA distribution and translation is achieved in both polarized and nonpolarized cells via long-distance transport and more local condensation of RNA molecules in ribonucleoprotein (RNP) granules. These granules are composed of numerous RNAs bound by regulatory RNA binding proteins (RBPs). My project aims at investigating the functional and molecular role of RBPs in vivo, using Drosophila nervous system as a model. Specifically, I study the binding properties of the wellconserved RBP Imp, which forms RNP granules transported to the axonal tips of neuronal cells. Inactivation of Imp in neurons leads to a loss of RNA transport along the axon and a developmental axon growth defect. Imp is composed of four RNA-binding domains called hnRNP-K Homology domain 1 through 4 (KH domains). These domains function in pairs (the KH1-2 and KH3-4 didomains) exhibiting different binding properties. To date, the contribution of the two di-domains in the selective recognition of cellular mRNAs and in the physiological function of Imp is unknown. To address these questions, we introduced point mutations in Imp KH domains that selectively disrupt RNA binding of KH12 or KH34 di-domains. The properties of these variants were tested both though in vitro binding experiments and by functional in vivo experiments. This revealed that KH1-2, but not KH3-4, is required for recruitment into RNP granules, and that KH34 di-domain is dispensable for neuron development. Interestingly, both di-domains are however required for fly viability, indicating that they have non-redundant, critical physiological functions. New experiments need now to be carried out to identify the respective targets of KH1-2 and KH3- 4 and their complementary roles in different Drosophila tissues. Altogether, this project will shed new light on the importance of domain modularity in RNA binding proteins.

THANK YOU FOR COMING

See you next year !

For any questions or any requests please contact us at :
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