26th of May 2016
Genopolys - Montpellier

14th meeting of PhD Students in Chemical and Biological Sciences for Health

Invited speaker
Damir Janigro (Pr, Cleveland Clinic Lerner College of Medicine)

CEO session
Vanessa Villard (Amylgen), Jérémy Dutheil (Neomerys),
Arnault loualalen (Numalis)
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Word from the CBS2 Association

It is with great pleasure that we receive you for the 14th annual meeting of the PhD students from the CBS2 Doctoral School: the CBS2 Day 2016!

Following the last years’ evolution, the Organizing Committee decided to strengthen this occasion for all the students to meet, talk and learn from each other and other members of the scientific community. Obviously, like last year, there will be 2 parallel Short Talks sessions to let you to choose the most interesting for you and 2 Poster sessions. But this year will be marked by the introduction of the Flash Talks to allow all PhD Students non selected for a Short Talk to do a flash oral presentation associated with their Poster.

We are also happy to receive Damir JANIGRO from the United States for a very interesting scientific communication bringing together basic research, clinic and innovative business foundation. The latest novelty this year is the integration of the outside academic world with the invitation of 3 PhD who founded their own company. Vanessa VILLARD, Jérémy DUTHEIL and Arnault IOUALALEN joined us to share their knowledge about alternative routes to academic research.

Once again, we would like to thank all the people who made it possible:
- Sandrine URVOY and Michel DESARMENIEN, head of our Doctoral School, supported us in our will of change,
- BioCampus with Matthieu RICHARD and Laurent JOURNOT, who made possible the reward for the best presentations,
- Genopolys with Magali KITZMANN, Géraldine PAWLAK, Silke CONQUET and Marcel MECHALI, who opened their doors to us,
- All the enthusiastic scientists constituting the jury and sharing with PhD students,
- Our partners, the University of Montpellier, MRI, IMGT, RHEM, Cisbio, Amylgen, Janvier Labs, Labex EpiGenMed, Pôle BioSanté Rabelais, CNRS, INSERM
- All of you who register in such large numbers to this event!

This meeting is originally organised by PhD students, for PhD students and is dedicated to the work we carry on every day in our labs. Enjoy the fact that this year more than 100 other members of the scientific community registered to the event to hear about the work done by PhD students.

Make this day your own!
# Program Overview

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<td>8h30-8h45</td>
<td>Welcome of the meeting attendees</td>
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<td>8h45-9h</td>
<td>Meeting opening by the Doctoral School director and the Asso CBS2 president</td>
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<td>9h-10h</td>
<td>3 Short Talks of PhD students for each session (in parallel)</td>
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<td>10h-10h30</td>
<td>Coffee break</td>
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<td>Conference of Damir Janigro</td>
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<td>(Pr. Cleveland Clinic Lerner College of Medicine)</td>
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<td>« Blood biomarkers in neurological diseases and their significance in neurobiology »</td>
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<td>11h30-12h</td>
<td>Flash Talks</td>
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<td>Poster Session 1 (with meal)</td>
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<td>13h45-14h45</td>
<td>3 Short Talks of PhD students for each session (in parallel)</td>
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<td>14h45-15h45</td>
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<td>15h45-16h15</td>
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<td>18h-18h15</td>
<td>Asso CBS2 Presentation</td>
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<td>18h15-18h30</td>
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<td>18h30-19h</td>
<td>Cocktail</td>
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Plan

Morning

Damir JANIGRO
Short Talks (Session 1A) | Flash Talks | Poster Session 1

★ Asso CBS2

☐ Cancer Biology

☐ Neuroscience

◇ Genetics, Epigenetics and Cell Determinism Control

☐ Infection and Immunity

△ Others:
- Experimental and Regenerative Medicine
- Biophysics, Structures and Systems
- Translational Medicinal Chemistry

Occitanie

Amphi 1
- Plenary Lecture
- Short Talks (Session 1A)
- Flash Talks

Reception

Poster Session
1 12
2 11
3 10
4 9
5 8
6 7
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15
16

Ground Floor
Morning

Damir JANIGRO (live broadcast)
Short Talks (Session 1B) | Poster Session 1

poster Session

2nd Floor
(1st Floor : COMUE Elections)
Afternoon

Vanessa VILLARD | Jérémy DUTHEIL | Arnault IOUALALEN
Short Talks (Session 2A) | Flash Talks | Poster Session 2

☆ Asso CBS2
○ Cancer Biology
□ Neuroscience
◊ Genetics, Epigenetics and Cell Determinism Control
☐ Infection and Immunity
▲ Others:
  Experimental and Regenerative Medicine;
  Biophysics, Structures and Systems;
  Translational Medicinal Chemistry

Occitanie

Amphi 1
Plenary Lecture
Short Talks (Session 2A)
Flash Talks

Ground Floor
Afternoon

Vanessa VILLARD | Jérémy DUTHEIL | Arnault IOUALALEN
(live broadcast)
Short Talks (Session 2B) | Poster Session 2

2nd Floor
(1st Floor : COMUE Elections)
Detailed Program

8h30-9h00: Welcoming

Words from Michel DESARMENIEN (Director of the CBS2 Doctoral School) and Benoit GIRARD (president of the CBS2 Association).

9h00-10h00: PhD Student’s Short Talks

Session 1A: Amphi 1 (Ground Floor)

9h00-9h20: HP1 are essential epigenetic regulators of liver homeostasis. Shefqet HAJDARI

9h20-9h40: DNA methylation dynamics of intestinal epithelial stem cells regulating colorectal cancer susceptibility and onset. Marco BRUSCHI

9h40-10h00: FOXD3+ trunk neural crest cells governs vertebrate appendage regeneration. Béryl LAPLACE-BUILHE

Session 1B: Amphi 2 (2nd Floor)

9h00-9h20: Intrathymic correction of a genetic immunodeficiency by AAV gene transfer. Marie POUZOLLES

9h20-9h40: H4-K20 methyltransferases in epigenetic mechanisms associated with cancer. Fanny IZARD

9h40-10h00: Development of a fluorescent peptide biosensor for probing CDK5 activity and monitoring the efficacy of new therapeutics in glioblastoma. Marion PEYRESSATRE

10h00-10h30: Coffee Break

10h30-11h30: Invited Speaker

Damir JANIGRO,
Cleveland Clinic Lerner College of Medicine and Flocel Inc. in USA.

“Blood biomarkers in neurological diseases and their significance in neurobiology”
11h30-13h30: Flash Talks and Poster Session 1 + Buffet

Poster 1 (with Flash Talk): Determinants for UBA1 recruitment at sites of DNA damage. Ramhari KUMBHAR

Poster 2 (with Flash Talk): Roles of EMT transcription factors in controlling cell clonal dynamics and invasiveness during emergence of tumor resistance in breast cancer subtypes. Emile LAKIS

Poster 3 (with Flash Talk): E4F1-mediated control of pyruvate dehydrogenase activity is essential for normal skin homeostasis. Berfin SEYRAN

Poster 4: Role of MDM2 for the tumor growth independantly of p53. Madi CISSÉ

Poster 5: Characterization of long-term protective immunity after anti-tumor-based immunotherapy in melanoma. Laetitia THEY

Poster 6: Regulation of RIP140 expression by the Wnt signaling pathway in colorectal and gastric cancer cells. Mouna TRIKI

Poster 7: Tuft cells function during intestinal tumorigenesis. Emmanuelle SIDOT

Poster 8: Modeling gene expression in cancers. May TAHA

Poster 9 (with Flash Talk): Oxytocin and vasopressin pace lateral septum electrical activity: consequences on brain rhythms and social memory. Amélie BORIE

Poster 10 (with Flash Talk): Functional expression of the NALCN ion channel in a neuronal cell line. Malik BOUASSE

Poster 11: Neural basis of motor control: study of the negative motor network with brain mapping. Fabien RECH

Poster 12: An herbicidal compound of the alpha-terthienyls class acts as a modulator of the pathological prion protein, PrPSc. Pierre-André LAFON

Poster 13: GPRIN1, a new 5-HT6 receptor partner that regulates receptor-operated Gs signaling and neurite growth. Camille PUJOL

Poster 14: Decoding of a new molecular mechanism in the regulation of early autophagic steps. Aurora SCRIVO

Poster 15: Generation of Alzheimer's disease (AD) genetic patients’ reprogrammed stem cells (iPS) as tools for the study of AD physiopathology. Laura AUBOYER

Poster 16: Gene therapy on rat models of the peripheral neuropathy Charcot-Marie-Tooth. Helene HAJJAR
Poster 17 (with Flash Talk): The role of the mechanosensitive ionic channels during zebrafish heart regeneration and development. Nathalie NASR

Poster 18 (with Flash Talk): A rat-tail model for bone regeneration and implant studies. Matthieu RENAUD

Poster 19: Fluorescent protein tag interrupts complete switching of the bacterial flagellar motor. Minyoung HEO

Poster 20: TGFB1: a potential regulator of cartilage homeostasis deregulated in osteoarthritis. Maxime RUIZ

Poster 21 (with Flash Talk): Role of the newly discovered XPR1 phosphate exporter in phosphate regulation and calcification. Uriel LÓPEZ SÁNCHEZ

Poster 22 (with Flash Talk): Massive redeployment of PRC1 proteins suppresses tumor formation during Drosophila development. Vincent LOUBIÈRE

Poster 23: Glutaminolysis and FAO regulate the relative commitment of HSCs to myeloid and erythroid lineage fates. Manuela ROMANO

Poster 24: Exploring the molecular grounds of genomic instability in early embryos. Elena LO FURNO

Poster 25: Characterization of the signaling pathway of an enzyme of epigenetic path H4-K20me. Nabiya BOUBACAR ALI

Poster 26: Gene SNPing of multiple genes involved in BCAA metabolism revealed their implication in obesity in French population. Sara HAYDAR

Poster 27 (with Flash Talk): HIV-1 envelope-induced autophagy is inhibited by virion incorporated Vpr in infected CD4 T cells. Jamal ALFAISAL

Poster 28 (with Flash Talk): Infection of human placental cells by Brucella strains causing abortion in baboons. Karellaen GARCÍA

Poster 29 (with Flash Talk): The indirect impact of immune-complexed adenovirus on dendritic cells. Tran THI THU PHUONG

Poster 30: Development of a multi-epitope peptide vaccine against human leishmaniasis. Joana PISSARRA

Poster 31: Analysing the role of secretion systems in the physiology and virulence of Brucella. Elia RIQUELME

Poster 32: Function of IL-10 producing B cells in healthy subjects and patients with rheumatoid arthritis and Sjögren's syndrome. Julie MIELSE
Poster 33: Characterization of early events of HIV-1 entry into dendritic cells and their impact on viral transmission. Laure PAPIN

Poster 34: Molecular epidemiology reveals genetic diversity amongst 363 isolates of the Cryptococcus neoformsans and C. gattii species complex in Ivorian HIV positive patients. Kassi KONDO FULGENCE

Poster 35: Dynamics of a T cell mediated autoimmune attack to the pancreas in real time. Gabriel ESPINOSA CARRASCO

**13h45-14h45: PhD Student’s Short Talks**

**Session 2A: Amphi 1 (Ground Floor)**

13h45-14h05: Role of the transcriptional coregulator RIP140 in hereditary colorectal cancer. Pascale PALASSIN

14h05-14h25: A real time, single molecule view of transcription and measurement of polymerases elongation rate in living cells. Alja KOZULIC PIRHER

14h25-14h45: The global regulator ShvR of Burkholderia cenocepacia tightly regulates virulence in zebrafish. Margarida CASTRO GOMES

**Session 2B: Amphi 2 (2nd Floor)**

13h45-14h05: The RON complex of T.gondii is injected during invasion into the host cell to bind the cytoskeleton. Amandine GUERIN

14h05-14h25: Physiological role of APPL in Drosophila mushroom body development. Claire MARQUILLY

14h25-14h45: The Gemini faces of Cyclin D1 in cancer cell apoptosis. Julien CHAMPAGNE

**14h45-15h45: PhD and CEO Invited Speakers**

Vanessa VILLARD (Amylgen)

Jérémie DUTHEIL (Neomerys)

Arnault IOUALALEN (Numalis)

**15h45-17h45: Flash Talks and Poster Session 2 + Coffee Break**
Poster 36 (with Flash Talk): Integrated approach of gamma-irradiation on Caenorhabditis elegans: from DNA to proteins. *Cécile DUBOIS*

Poster 37 (with Flash Talk): L type Cav1.3 and T-type Cav3.1 calcium channels in cardiac pacemaker activity. *Matthias BAUDOT*

Poster 38 (with Flash Talk): Interaction of Fe3O4@MSN nanoparticles with cells membranes. *Estelle RASCOL*

Poster 39: Screening and vectorization of therapeutic peptides for cystic fibrosis treatment. *Quentin SEISEL*

Poster 40: S100A10 silencing promoted apoptosis of epithelial cells resulting probably in implantation failure process. *Loubna DRISSENNEK*

Poster 41: Sarcoplasmic Reticulum Ca2+ leak and mitochondrial reactive oxygen species: the auto-amplification loop. *Mathilde LACÔTE*

Poster 42 (with Flash Talk): Regions 1.2 and 3.2 of the RNA Polymerase σ Subunit Promote DNA Melting and Attenuate Action of the Antibiotic Lopiarmycin. *Zakia MORICHAUD*

Poster 43: Role of miRNA in Cystic Fibrosis pathology. *Alexandra POMMIER*

Poster 44: Identification of epigenetic predictive biomarkers of lung disease severity in cystic fibrosis. *Fanny PINEAU*

Poster 45: Studying the Function(s) of Obi1, a Novel ORC Ubiquitin Ligase, using Xenopus Egg Extracts. *Joelle NASSAR*

Poster 46: Risks of in utero Non Steroidal Anti-Inflammatory Drugs and acetaminophen exposure on the early testis development. *Moïra ROSSITTO*

Poster 47 (with Flash Talk): A good way to increase the efficiency of rescuing virus. *Haijin LIU*

Poster 48 (with Flash Talk): Behavior of a new colonizing S. aureus strain in stress conditions. *Christelle NGOBA ESSEBE*

Poster 49 (with Flash Talk): Enhancing adoptive immunotherapy: redirecting immune subsets and metabolic pathways. *Carmen YONG*

Poster 50: Cell surface Glut1 levels distinguish human T lymphocyte subsets with distinct effector functions. *Maria MATIAS*

Poster 51: Identification and characterisation of a new Coxiella burnetii effector that participate to vacuole biogenesis. *Fernande AYENOUE SIADOSU*

Poster 52: Role of nitrosative stress in the anti-mycobacterial properties of the KdpF membrane peptide. *Mariana ROSAS OLVERA*

Poster 53: The response to extreme acid stress in Brucella: role of the genes in the gad locus. *Freddi LUCA*

Poster 54: EBI2 interacts with the HIV co-receptor CCR5 and enhances HIV infection. *Adeline GUIGUES*

Poster 55 (with Flash Talk): Contribution of CD8+ T cells in the pathophysiology of amyotrophic lateral sclerosis. *Emmanuelle COQUE*

Poster 56: Hetero-oligomerization of dopamine D1 and metabotropic glutamate mGlu5 receptors modulates cellular signaling. *Elise GOYET*

Poster 57: The human OPA1delTTAG mutation induces age-related auditory neuropathy in mouse. *Carolanne COYAT*

Poster 58: Tackling epileptogenesis via the mGlu7 glutamate receptor. *Benoit GIRARD*

Poster 59: Phosphoproteomics of 5-HT2A/mGlu2 heteromers: toward new insights into the mechanism of action of hallucinogens and antipsychotics. *Samy MURAT*

Poster 60: Spinal Cav3.2 channel deletion and pain sensibility. *Antoine FRUQUIERE*

Poster 61: Oxidative stress-induced p66 expression: key mechanism of age-related cochlear sensory hair cell loss. *Nesrine BENKAFADAR*

Poster 62 (with Flash Talk): TRIM proteins regulate ubiquitination and degradation of the anti apoptotic protein Bfl-1. *Loïc LIONNARD*

Poster 63 (with Flash Talk): The role of Cyclin A2 in colorectal carcinogenesis. *Yuchen GUO*

Poster 64 (with Flash Talk): Translational control in cancer cells. *Laura YAZDANI*

Poster 65 (with Flash Talk): Diffuse low grade gliomas: the elaboration of a new in vitro model and the study of the cancerous/stroma interactions. *Safa AZAR*

Poster 66: Modeling gene expression in cancers. *Chloé BESSIERE*

Poster 67: Combinatorial strategy targeting the tumor microenvironment of triple-negative breast cancer with anti-protease antibody. *Hanane MANSOURI*

Poster 68: Dissection of E4F1 function on cell cycle checkpoints and DNA repair in response to genotoxic stress. *Kalil BATNINI*
Poster 69: Crosstalk of the transcription factor RIP140 with the Notch signaling pathway in colon cancer. *Nour SFEIR*

Poster 70: Integrated models for assessment of significant biological mechanisms in oncopharmacology and aging: C. elegans and H. dujardini. *Myriam RICHAUD*

**17h45-18h15: Asso CBS2 Presentation**

General informations about the association and its projects.

**18h15-18h30: Award Ceremony**

To reward the best of you and win a large variety of gifts…

**18h30-19h00: Coktail**

And don’t forget the Party Tonight… !
Invited Speakers
**Damir JANIGRO**

« Blood biomarkers in neurological diseases and their significance in neurobiology »

Damir JANIGRO is professor and scientific director of a biotechnology company. His research work at the Cleveland Clinic Lerner College of Medicine are of various kind, going from the study of the neuro-vascular unit and its relations to the pathological functioning of the brain to the study of the neuronal control of the inflammation and the biomarkers of cerebral pathologies like epilepsy, cerebral lesions, Alzheimer, or schizophrenia. Music enthusiast, he has also been exploring its effect on the brain using deep cerebral electrodes. The main objective of his research is to understand the way the neuronal environment maintain bioelectric homeostasis in the brain and thus the physiological electric activity. His tight relations with the clinic field gives him the opportunity to keep the human in the center of his research.

The quality of his work has been supported by a permanent funding of the NIH for the past 20 years.

On the other hand, confronted to the absence of in vitro models for the study of the neuro-vascular unit, he decided to found Flocel, Inc, a company that deals with models of Blood Brain Barrier.
PhD and CEO
Vanessa VILLARD | Jérémy DUTHEIL | Arnault IOUALALEN

Vanessa VILLARD is a PhD in sciences. She graduated from the University of Montpellier. During her thesis, she contributed to the development of a predictive and validated model of Alzheimer’s Disease. In 2009, in collaboration with her thesis supervisor, she found a company: Amylgen. The company offers to industry to evaluate on in vivo models the therapeutic potential of new molecules in the neurosciences field. In perpetual search of performance, Vanessa Villard developed and complemented her technical abilities accordingly with her ambition.

Jérémy DUTHEIL is a PhD in Sciences. He graduated from the Paris Sud University. His thesis project was based on the study of the metabolism of the photoproduction of hydrogen by a cyanobacteria. He thinks about an alternative and original way of producing energy by using biotechnologies. The process reverts the greenhouse gases emission and is thus carbonegative. Jérémy Dutheil founded Néomérys to develop a panel of technologies that allows the production of a lot of biofuel at low cost. He converted his knowledge and used his research to make his company grow.

Arnault IOUALALEN is a PhD in informatics and research engineer in mathematics. He’s developing a project to enhance the accuracy of softwares and founded his company: Numalis. His philosophy is to combine the better of both the academic and industrial world. He’s recruiting and making people exchange the knowledge and point of view of both worlds. Throughout his projects, Arnault IOUALALEN introduces the philosophy of entrepreneurship and promotion of the academic knowledge.
Short Talks - Session 1A
Amphi 1
Chromatin plays essential roles in cell identity and cancer development and its dynamics is highly regulated by chromatin associated proteins such as HP1. We show that inactivating the genes encoding HP1α and HP1γ in mice predispose mice to develop tumors specifically within liver. Furthermore, specific inactivation of HP1β and HP1γ within hepatocytes also significantly increases the incidence of tumor, suggesting that HP1 are liver specific tumor suppressors. Histological analysis of HP1αβliverKO livers, revealed defects that resembled those observed in human liver pathologies known nonalcoholic steatohepatitis (NASH) characterized by an increase of steatosis, followed by an increased inflammation and the development of fibrosis that ultimately leads to tumors in old animals. In the case of HP1αγliverKO mice, although inflammation and tumour development were observed, this was not linked with steatosis, strongly suggesting that although HP1s are important within hepatocytes for liver homeostasis the underlying mechanisms are specific of each HP1 isoform. Analysis of gene expression in young animals revealed that the genes up-regulated in HP1αβliverKO and HP1αγliverKO livers are strongly enriched in genes encoding several members of the family of transcriptional repressors known as the KRAB-ZFPs. KRAB-ZFPs act through the recruitment of the TRIM28 corepressor which interacts with HP1 for some but not all of its functions. Using mice expressing a TRIM28 protein unable to interact with HP1 specifically within hepatocytes, we demonstrated here that the disruption of the interaction between TRIM28 and HP1 lead to spontaneous development of tumors within liver and to over-expression of the same KRAB-ZFP as those deregulated in HP1αβliverKO and HP1αγliverKO mice. Altogether, our data demonstrated that HP1 are liver-specific tumor suppressor. They also suggest that HP1 main function within liver is to regulate TRIM28 activity and thereby regulate the expression and repression activity of KRAB-ZFP and ultimately liver homeostasis.

Shefqet HAJDARI, Florence CAMMAS
Cancer initiation and progression represent the outcome of the progressive accumulation of genetic and epigenetic alterations. Global changes in the epigenome are now considered as a common hallmark of malignancies. However, most of our present knowledge represents the result of the comparison between fully established malignancies and their surrounding healthy tissue. Such comparison is not informative about the epigenetic contribution to the very early steps of cancer onset. By making use of innovative strategies and relevant in vivo models we aim at shedding light on the correlation between the interindividual epigenetic polymorphisms within the population and the relative risk to develop malignancies, and establish the existence of an epigenetic signature associated with an increased susceptibility to intestinal cancer. We also investigated in vivo the timing at which the early remodeling occur at the epigenomic scale by analyzing the alterations in the DNA methylation and gene expression profiles upon the loss of the Apc gene, the most common genetic lesion associated with colorectal cancer initiation in human.

Our results confirm that a considerable degree in the variability associated to cancer susceptibility cannot be ascribed to major genetic changes and that such heterogeneity correlates with an epigenetic signature. We also found that the loss of function of Apc in the intestinal Lgr5+ stem cell compartment is rapidly accompanied by a reprogramming of the DNA methylation profiles resulting in altered gene expression and impaired fate determination in those cells.

Overall, this indicates that the epigenetic remodeling is an early event in tumorigenesis that might even precede actual cell transformation. The functional implications of our results in cancer development are currently under investigation.

Marco BRUSCHI¹, Laure GARNIER¹, Sarah BAHRAOUI¹, Miloud ZENATI¹, Michael WEBER², Philippe JAY¹. ¹Institut de Génomique Fonctionnelle, Montpellier. ²Ecole supérieure de biotechnologie de Strasbourg.
**N°3 - Béryl LAPLACE-BUILHE**

**FOXD3+ trunk neural crest cells govern vertebrate appendage regeneration**

Introduction: Zebrafish displays an outstanding potential to regenerate many tissues including the caudal fin. This process relies on the formation of a highly proliferative structure called the blastema described as a pool of highly proliferative stem/progenitor cells. Although critical for the regenerative process and intensively studied this structure remains poorly defined. Neural crest cells (NCCs), a vertebrate innovation, represent a transient population of cells with extended differentiation capacities that forms during neurulation and migrate over long distances to form a wide range of tissues. In the present study, we address the role of trunk NCCs in blastema formation and caudal fin regeneration.

Materials, Methods and Results: Using confocal microscopy and a double transgenic line Tg(Foxd3:GFP/rcn3:mCherry) to track in green NCCs and in red mesenchymal cells (MCs) we observed the presence of Foxd3+ NCCs, RNC3+Foxd3- MCs and RNC3+Foxd3+ MCs within the regenerating caudal fin of zebrafish larvae. Live imaging analysis revealed morphological and behavioral changes of these cell types during the caudal fin regeneration process and interactions between Foxd3+ NCCs and RNC3+MCs. Focusing on cell proliferation events, we found that RNC3+ MCs proliferate only when they were in contact with Foxd3+ NCCs suggesting a role of Foxd3+ NCCs on MC proliferation and blastema formation. Then, using the morpholino-mediated Foxd3 silencing technique we demonstrated that Foxd3+ NCC depletion dramatically impaired MC proliferation, blastema formation and fin regeneration.

Conclusion: Altogether our results unveil the pivotal role of NCCs in blastemal formation, providing new insights into the mechanisms of appendage regeneration.

*Laplace-Builhe B.1,2, Luz-Crawford P.1,2, Tejedor G.1,2, Riquier S.1,2, Mathieu M.1,2, Nguyen-Chi M.1,2, Jorgensen C.1,2,3, Djouad F.1,2.* 1Inserm U1183, IRMB, Montpellier, France 2Université Montpellier, France 3CHU Lapeyronie, Montpellier, France.
Short Talks - Session 1B
Amphi 2
Intrathymic correction of a genetic immunodeficiency by AAV gene transfer

Patients with combined immunodeficiency can be cured by allogeneic hematopoietic stem cell transplantation but complications often occur if the donor is incompatible. To circumvent these problems, significant efforts have been invested in the development of gene therapy strategies. Nevertheless, adverse events indicate the necessity of exploring other avenues. Our group hypothesized that in situ gene correction of T lymphoid progenitors in the thymus itself may overcome some of the drawbacks of ex vivo gene correction.

As lentiviral gene transfer in the thymus is minimal, we assessed the potential efficacy of adeno-associated virus (AAV) serotypes for thymocyte transduction. Intrathymic administration of scAAV2/8, 2/9 and 2/10 vectors resulted in a >10-fold higher transduction of thymocytes (3-5%) as compared to lentiviral vectors with rapid transgene expression, detected by three days post injection. scAAV2/8 vector promoted the highest level of transduction and strikingly, transduced cells represented up to 1% of peripheral T lymphocytes following scAAV2/8 gene transfer, even in immunocompetent mice. In the context of ZAP-70-/- immunodeficient mice, we found that intrathymic injection of an AAV2/8-ZAP-70 vector resulted in a rapid and efficient T cell reconstitution. Furthermore, stable expression of a functional ZAP-70 transgene (> 4 months) allowed functional T cell responses. Thus, AAV vectors, even though they persist mainly as vector episomes, are maintained long-term and can be used for stable in vivo transgene expression in the thymus, promoting T cell function.

Pouzolles M.1, Gailhac S.1, Adjali O.2, Moullier P.2, Taylor N.1 and Zimmermann V.S.1

1IGMM, 1919 route de Mende, 34293 Montpellier cedex 5 (France). 2UMR1089, 8 quai de Moncousu, BP70721, 44007 Nantes (France).
marie.pouzolles@igmm.cnrs.fr
Assembled with DNA to form nucleosomes, histone proteins are the basic building blocks of chromatin. A striking feature of histones is that they are subject to a large number of post-translational modifications, including phosphorylation, acetylation, and methylation. These histone modifications are thought to contribute to the regulation of all DNA-templated processes by mediating both alterations in chromatin structure and recruitment of non-histone proteins to specific regions of the genome. Lysine methylation is one of the most intriguing histone modifications in view of its remarkable complexity. It is detected on many histone lysines, each of which can be mono-, di-, or tri-methylated. These modifications occur at highly conserved positions and often cluster within specific regions to organize chromosomes into structural and functional domains.

PR-Set7 is the sole enzyme responsible for the monomethylation of histone H4 at lysine 20, which is required for subsequent catalysis of H4-K20 di- and tri-methylation via the activity of SUV4-20h enzymes. Loss and gain-of function experiments have shown that the concerted activity of H4-K20 enzymes is essential for the regulation of chromatin structure, DNA replication and repair, thereby establishing this chromatin-signaling pathway as central in the maintenance of genome integrity. In line with this, tumors are often characterized by alterations in levels of different H4-K20me states, which are suspected to play a role in both genesis and progression of cancer cells. The main objective of my thesis is to unravel the functions of H4-K20 enzymes and their marks in the regulation of chromatin structure. Using high-resolution microscopy approach I have shown that PR-Set7 overexpression (as observed in cancer) led to chromosome alterations, including abnormal chromatin compaction and changes in localization of heterochromatin marks (H3K9me3, HP1α, SMC3). I will determine how alterations in the activity of these enzymes contribute to tumorigenesis and the link with chromatin structure alterations.

Fanny Izard, Charlotte Grimaud, Eric Julien
N°6 - Marion PEYRESSATRE
Development of a fluorescent peptide biosensor for probing CDK5 activity and monitoring the efficacy of new therapeutics in glioblastoma

Confidential
Short Talks - Session 2A
Amphi 1
Role of the transcriptional coregulator RIP140 in hereditary colorectal cancer

Colorectal cancer (CRC) is a common disorder with familial forms such as Lynch syndrome which exhibit microsatellite instability (MSI) due to loss of function of DNA mismatch repair system (MMR). However, some clinically diagnosed families do not exhibit MMR gene alterations (Lynch Like Syndrome or LLS) thus implicating new candidate genes. We recently reported that the transcription factor RIP140 is involved in sporadic colorectal carcinogenesis. A frame shift mutation in the RIP140 coding sequence (RIPMSI) which generates a truncated protein has been detected in cells and tissues from MSI CRC. This suggested that RIP140 and its mutation might be linked to microsatellite instability associated with familial CRC.

The role of RIP140 on the regulation of MMR gene expression was investigated by using different human colorectal and murine cell lines displaying a deregulated expression of RIP140. It appears that RIP140 regulates the expression of several MMR genes both at the mRNA and protein levels. In addition, RIP140 mRNA expression was found significantly correlated with MMR genes in a cohort of CRC patients (p<0.001). To define the functional consequences of these regulations, the effect of RIP140 expression on cell sensitivity to different cytotoxic drugs was first studied. RIP140 expression was associated with an increased resistance to several drugs including oxaliplatin, 5-fluorouracil and SN38. The microsatellite stability status of different models will be analyzed in cells with altered RIP140 expression. In parallel, the sequencing of tumor DNA from 93 MSI CRC patients has confirmed the presence of the RIPMSI mutation in about 15% of the cases. In conclusion, by decreasing the expression of genes implicated in maintenance of genome integrity, the RIPMSI mutation might explain microsatellite instability in patients where no MMR gene mutation is found.

Pascale PALASSIN, Marion LAPIERRE, Carole CORSINI, Sandrine BONNET, Stéphan JALAGUIER, Audrey CASTET-NICOLAS, Vincent CAVAILLES. IRCM, INSERM U1194, Montpellier.
A real time, single molecule view of transcription and measurement of polymerases elongation rate in living cells

Transcription is a fundamental step in the gene expression. However, how this process occurs in live cells and real time is still not well characterized. Here, we improved the RNA tagging system, using an HIV reporter. The reporter was inserted in HeLa cells and consisted of an HIV promoter fused to 128xMS2 stem-loops. On each stem loop two MS2 coat proteins fused with GFP can bind with high affinity. This allows a quantitative, single molecule view of transcription in real time. However, despite all these improvements, the measurements of polymerase elongation rate are still not precise. Therefore, our main focus was to develop a two-color system. Indeed, by introducing a second RNA tag, visible in another color, we are able to detect two independent signals that occur at different time points and that can used to directly calculate the velocity. Furthermore, by using the simulation and modeling we can determine the exact position of the polymerases throughout the movie and transcriptional cycles. We intend to use this system to evaluate: (i) the impact of the sequence composition on polymerase elongation rate; (ii) the link between splicing and the velocity of polymerases; (iii) the impact of the promoter on elongation.

Kozulić-Pirher A., Müller F., Gostan T., Zimmer C., Basyuk E., Bertrand E.

1Institut de Génétique Moléculaire de Montpellier; CNRS – UMR5535; 1919, route de Mende; 34293 Montpellier Cedex 5; France. 2Unité Imagerie et Modélisation, Institut Pasteur and CNRS UMR 3691; 28, rue du Docteur Roux; 75015 Paris.
N°9 - Margarida CASTRO GOMES

The global regulator ShvR of Burkholderia cenocepacia tightly regulates virulence in zebrafish

Pulmonary infections with bacteria belonging to the Burkholderia cepacia complex (Bcc) are known to worsen clinical outcome for cystic fibrosis (CF) patients. How persistent bacteria can cause sudden periods of pulmonary exacerbation, sometimes resulting in rapidly fatal necrotising pneumonia and septicaemia (Cepacia Syndrome), is not known. B. cenocepacia has been shown to be capable of surviving and multiplying inside host cells in vitro. In our laboratory, we have shown for the first time in vivo, using a zebrafish infection model, that macrophages provide a critical site for intracellular replication of B. cenocepacia K56-2 and development of acute pro-inflammatory infection. Although it is generally believed that Bcc bacteria are present in biofilms in infected lungs, a role for macrophages and bacterial factors in intracellular survival or induction of acute pro-inflammatory infection is not known.

The LysR-type transcriptional regulator ShvR of B. cenocepacia K56-2 has been shown to regulate genes important for virulence in a rat model of infection. Although the absence of shvR resulted in strongly reduced lung inflammation, this, paradoxically, correlated with higher bacterial loads. This suggests that the shvR mutant was highly persistent. We used zebrafish larvae to further study a role for ShvR during intracellular stages and for the induction of acute fatal inflammation. We found that the absence of shvR resulted in a striking reduction in pro-inflammatory responses and tissue inflammation preventing fatal infection. Using real time non-invasive imaging we observed that shvR mutant bacteria were able to persist in macrophages but, in contrast to the wild type parent strain, were unable to disseminate from infected macrophages. We will present how we used an inducible reporter system, to show in vivo that ShvR tightly regulates genes needed for the development of acute pro-inflammatory infection.

Margarida Gomes¹,², Sujatha Subramoni³, Pamela Sokol³ and Annette Vergunst¹,². 
¹INSERM, U1047, UFR Médecine Site de Nîmes, 30908 Nîmes, France. ²Université de Montpellier, U1047, UFR Médecine, 30908 Nîmes, France. ³Department of Microbiology, Immunology, and Infectious Diseases, University of Calgary, Calgary T2N 4N1, Canada.
Short Talks - Session 2B
Amphi 2
Toxoplasma gondii is an obligate intracellular parasite which belongs to the Apicomplexa phylum, including Plasmodium species. The invasion mechanism is unique among this phylum. It involves the formation of a tight connection between the parasite and the host cell plasma membranes called moving junction (MJ). During invasion, a complex composed of the parasite rhoptry neck proteins RON2/4/5/8 is injected into the host cell and localizes to the MJ. RON2 spans the host plasma membrane and functions as a receptor for the microneme protein AMA1 exposed on the parasite surface. It results in a close and irreversible contact between the parasite and the host cell. RON4, RON5 and RON8 are exposed on the cytosolic face of the host cell.

The contribution of the host cell in invasion remains a major enigma in the field. While located at the host cytosolic face of the MJ, the mechanistic role of the RON4/5/8 proteins during invasion remains elusive. We hypothesize that the complex could bind host proteins and anchor the MJ to the cell cortex to sustain parasite invasive force.

Here, we present the identification of ALIX and TSG101 as host partners of respectively Toxoplasma RON4 and RON5. We demonstrated that theirs recruitments at the MJ during T. gondii tachyzoite invasion are RON dependent and we have identified theirs binding domains on RON4 and RON5. Both ALIX and TSG101 are adaptors proteins involved in the ESCRT machinery and are known to be hijacked by some viruses for the budding process. ALIX is also known to bind actin, α-actinin and cortactin, which is particularly interesting in the light of modification of dynamic of host cytoskeleton during invasion. We propose that RON proteins recruit these host partners at the MJ to play critical functions for invasion as anchoring the junction to the actin cortex.

Amandine Guérin¹, Maude Lamarque¹, Michelle Parker², Rosa Milagros Corrales¹, Orly Salama-Alber², Martin Boulanger² and Maryse Lebrun¹. ¹UMR 5235 CNRS, Université de Montpellier 2, 34095 Montpellier, France. ²Department of Biochemistry & Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 3P6.
The protein APPL (Amyloid Precursor Protein-Like) is the Drosophila homologue of human APP known to be involved in Alzheimer’s disease (AD). Despite its involvement in AD, the normal physiological role of APP is still poorly understood. We are investigating the role of APPL in brain development. APPL is highly expressed in the mushroom bodies (MBs), which are the Drosophila centers for learning and memory. Recently, APPL was described as a novel neuronal-specific modulator of the PCP pathway required for the robustness of axonal outgrowth of the MB during development (Soldano et al. PlosBiol 2013). It was shown that this protein facilitates the PCP-specific phosphorylation of the Wnt adaptor protein DSH (dishevelled) by the Abelson kinase (Abl). We show here that the Drosophila homologue of the human protein Huntingtin (HTT), involved in Huntington’s disease (HD), likely also regulates this PCP pathway. Indeed, we find that the MB mutant phenotype caused by the loss of APPL is suppressed when one dose of htt is removed or when htt-targeting RNAi is specifically expressed in the MBs. Our other results indicate that the target of HTT could be ABL. We are currently testing the hypothesis that, while the ABL kinase is activated by APPL, it is directly inhibited by the HTT protein in order to insure a balanced and controlled level of activation of the axonal outgrowth pathway. If confirmed, this will demonstrate a profound functional interaction between two critical human disease genes, both central players in neurodegeneration, and an important proto-oncogene that has key roles in development and cancer.

Claire Marquilly¹, Lee G. Fradkin², Jean-Maurice Dura¹. ¹Institut de Génétique Humaine, Montpellier. ²UMass Medical School, Department of Neurobiology, Worcester.
Cyclin D1 is a major component of the core cell cycle machinery that activates the CDK4/6 kinases in G1 phase. Despite retinoblastoma protein (RB) inhibition via CDK4 activation, the kinase independent role of Cyclin D1 appears also to contribute largely to cancer development. Using high throughput genomics and proteomics approaches, we addressed the contribution of Cyclin D1 in tumor maintenance. Thanks to a unique gene silencing tool based on RNA interference that we named TAG-RNAi, we found that the specific targeting of Cyclin D1 is sufficient to induce tumor regression in vivo and to enhance cancer cell responsiveness to chemotherapy in vitro. Interestingly, our proteomics screening revealed a dynamic interaction between Cyclin D1 and Caspase 3 after cell stress. Since Caspase 3 is a major apoptosis executioner, we found by deep molecular investigations, that Cyclin D1 acts both as an activator and inhibitor of programmed cell death. Our results illustrate that Cyclin D1 favors the formation of inactive Caspase 3 dimers, while it inhibits their activation by upstream activator Caspases 8 and 9. Therefore, by modulating Cyclin D1 threshold at the genetic or mRNA level, we found that it is possible to use Cyclin D1 as a synthetic lethality support for adjuvant chemotherapy by RNA interference. Interestingly, this new molecular action of Cyclin D1 is independent of CDK4 kinase activity but remains connected to CDK4 protein which appears to stabilize Cyclin D1 half-life. Consequently, Cyclin D1, CDK4 and Caspase 3 abundance may reflect informative biomarkers of responsiveness to chemotherapy and we propose to monitor these proteins from limited biological material using a novel Tandem-HTRF approach.

Julien Champagne\textsuperscript{1}, Benjamin Maurel\textsuperscript{1}, Alexandre Zampieri\textsuperscript{1}, Laetitia K. Linares\textsuperscript{2}, Ivanna Fuentes\textsuperscript{1}, Emeric Dubois\textsuperscript{3}, Dany Severac\textsuperscript{1}, Clément Assailli\textsuperscript{4}, Martial Seveno\textsuperscript{4}, Frédéric Bienvenu\textsuperscript{1}. \textsuperscript{1}IGF. \textsuperscript{2}ICM. \textsuperscript{3}MGX Montpellier Genomix. \textsuperscript{4}Functional Proteomic Platform Montpellier.
Flash Talks and Poster Session 1
Determinants for UBA1 recruitment at sites of DNA damage

Ubiquitylation is an important posttranslational modification that is necessary for protein degradation as well as for the regulation and the localization of many cellular factors. A number of proteins implicated in DNA replication and DNA damage response are ubiquitylated. Ubiquitylation during the DNA damage response is selectively dependent on the ubiquitin-activating enzyme UBA1, which functions at the apex of the ubiquitylation cascade. Our objective is to elucidate whether and how UBA1 is recruited at damaged sites and to uncover the role of ubiquitylation in ATR signaling.

Using a cell free system developed in the lab that recapitulates ATR kinase-signaling pathway, we found that UBA1 is recruited to linear DNA substrates and mediates ubiquitylation of DNA-bound proteins. ATR-mediated Chk1 phosphorylation in cell-free extracts was dependent on UBA1 activity. Intriguingly, upon UBA1 inhibition, Chk1 accumulated on biotinylated DNA coupled to streptavidin beads. We found that protein ubiquitylation and the recruitment of UBA1 to DNA in cell-free extracts was dependent on the kinase DNA-PKcs and on the poly ADP-ribose polymerase PARP1, two sensors of DNA lesions. UBA1 exhibited affinity for PARP1 and for poly ADP-ribose (PAR) chains in vitro. Consistent with this, PARP1 promoted UBA1 recruitment at an inducible DNA double-strand break in living cell, as revealed by chromatin immunoprecipitation. Furthermore, we have identified hydrophobic residues in disordered region of UBA1 which are required for its PAR binding activity. Using protein replacement system we demonstrated that mutation in PAR binding residues impairs DNA damage response.

These results indicate that UBA1 is recruited to DNA damaged sites in a DNA-PKcs and PARP1 dependent-manner and that protein ubiquitylation is necessary for the assembly of a productive ATR signaling complex. Thus, UBA1 inhibitors could be used to target ATR signaling in cancer cells.

Ramhari Kumbhar¹, Sophie Vidal-Eychenie¹, Alkmini Kalousi², Evi Soutoglou², Cyril Ribeyre¹ and Angelos Constantinou¹. ¹Institute of Human Genetics (IGH), CNRS UPR-1142, Montpellier France. ²Institute of Genetics and Molecular and Cellular Biology (IGBMC), Strasbourg France.
Roles of EMT transcription factors in controlling cell clonal dynamics and invasiveness during emergence of tumor resistance in breast cancer subtypes

This project explores the mechanisms of mammary gland morphogenesis, as a model for breast carcinoma progression. Mammary gland morphogenesis results from the coordination of distinct cell responses (proliferation, differentiation, motility, invasiveness, apoptosis) integrated by numerous pathways, including Wnt, EGF, FGF, Notch, SHH, Myc and hormonal activation. For the purpose of this study, we feel it is critical to analyze individually the impact of these pathways in modulating proliferation, differentiation, motility, invasiveness, apoptosis, intercellular cohesion, and polarity in cells involved in a coherent morphogenetic migration.

We have designed improved 3D models to analyze the impact of EMT-TF in a 3D environment. Our system allows monitoring simultaneously the mentioned pathways at a cellular level for three weeks, a period adjusted to test chemotherapy drugs.

Our first model describes the primary emergence of invading breast carcinoma cells from mammary epithelium. Cells are treated with defined drugs or will be transfected with various constructs (under validation) enhancing or repressing specific pathways such as Slug, in addition to constructs allowing the monitoring of cell structures by GFP labeling for video microscopy.

LAKIS Emile, BONINI Fabien, SAVAGNER Pierre. IRCM (INSERM-U1194)
The multifunctional protein E4F1 is an essential regulator of epidermal stem cell (ESC) maintenance. Here, we found that E4F1 transcriptionally regulates a metabolic program involved in pyruvate metabolism that is required to maintain skin homeostasis. E4F1 deficiency in basal keratinocytes resulted in deregulated expression of dihydrolipoamide acetlytransferase (Dlat), a gene encoding the E2 subunit of the mitochondrial pyruvate dehydrogenase (PDH) complex. Accordingly, E4f1 knock-out (KO) keratinocytes exhibited impaired PDH activity and a redirection of the glycolytic flux towards lactate production. Metabolic reprogramming of E4f1 KO keratinocytes associated with remodelling of their microenvironment and alterations of the basement membrane, leading to ESC mislocalization and exhaustion of the ESC pool. ShRNA-mediated depletion of Dlat in primary keratinocytes recapitulated defects observed upon E4f1 inactivation, including increased lactate secretion, enhanced activity of extracellular matrix remodelling enzymes, and impaired clonogenic potential. Altogether, our data reveal a central role for Dlat in the metabolic program regulated by E4F1 in basal keratinocytes and illustrate the importance of PDH activity in skin homeostasis.


*equal contribution #co-senior authors
N°4 – Madi CISSÉ
Role of MDM2 for the tumor growth independantly of p53

Confidential
N°5 – Laetitia THEY
Characterization of long-term protective immunity after anti-tumor-based immunotherapy in melanoma

My research PhD project aims at a better understanding of the relationship between cancer cells and the immune system, with the specific objectives to i) study the contribution of immune effectors in tumor control after monoclonal antibody (mAb)-based anti-tumor immunotherapies and ii) optimize the use of these mAbs trough development of combined therapies to set up long-term anti tumor effects. Using the B16F10 melanoma mouse model, the team showed that an immunotherapy based on the use of TA99 mAb (directed against the TYRP1 antigen overexpressed on tumor melanocytes), significantly increases mice survival with 75% of mice showing a significant delay in tumor progression and 25% of mice showing no tumor development at all. Based on these results, the first objective of my PhD project is to characterize the contribution of endogenous immunity in the long-term protection effects observed in TA99-treated mice.

The first results showed that when tumor-free mice received a second graft with B16F10 cells (challenge), 50% of them did not develop any tumor after the challenge suggesting the presence of a specific anti-tumor immunity. Analysis of the presence of specific anti-tumor cell immunoglobulins within the sera of protected mice by ELISA, showed an increase of the humoral response after challenge. Moreover, my preliminary results indicated that transfer of serum from challenged-protected mice into naïve B16F10-grafted mice delayed tumor growth. Such results suggest the contribution of the humoral response in the long-term protective effects mediated by TA99 immunotherapy. Experiments are now in progress to further characterize these effects and to analyze the contribution of the cytolytic immune response in TA99 immunotherapy.

Laetitia They, Henri-Alexandre Michaud, Virginie Lafont, Jean-François Eliaou, Laurent Gros*, Nathalie Bonnefoy*
*Co last authors
Regulation of RIP140 expression by the Wnt signaling pathway in colorectal and gastric cancer cells

Gastrointestinal cancer such as colon (CRC) and gastric (GC) cancers are the main causes of death worldwide. They arise through multistep processes in which genetic and epigenetic alterations accumulate in a sequential order. The aberrant activation of the Wnt/β-catenin signaling pathway is involved in the development and progression of such cancers. Recently, it has been shown that the transcriptional coregulator RIP140 is involved in sporadic colorectal carcinogenesis. In the intestinal epithelium, RIP140 regulates cell proliferation by inhibiting the Wnt signaling pathway. It has been also revealed that RIP140 expression decreased during tumorigenesis at both the mRNA and protein levels. Here we have investigated the regulation of RIP140 gene expression by the Wnt signaling pathway in colorectal and gastric cancer cells.

The effect of Wnt signaling on RIP140 expression in CRC and GC cells was tested by qRT-PCR and transient transfection. Our preliminary results showed that Wnt signaling activation by LiCl or SB216763 inhibits RIP140 mRNA accumulation. In transient transfection experiments, activation of the Wnt pathway by LiCl and through active β-catenin ectopic expression inhibits transcription of the human and mouse RIP140 promoters suggesting that this downregulation occurs at the transcriptional level. On the other hand, using RIP140 promoter deletion mutants in transient transfection, we have confirmed that this regulation implicates the proximal region of RIP140 promoter.

The downregulation of the transcriptional coregulator RIP140 gene by Wnt/β-catenin has been validated on both colon and gastric cell lines, yet the mechanisms of this regulation are still to be defined (point mutation in the proximal promoter region, ChIP analysis…). Further experiments are also needed to determine whether cross talk between other signaling pathways could play a role in this regulation.

Mouna TRIKI1,2*, Sandrine BONNET1, Raja GARGOURI2 et Vincent CAVAILLES1.

1IRCM, Institute of Cancer Research of Montpellier, INSERM U1194, Montpellier University, Montpellier, France. 2Center of Biotechnology of Sfax, Laboratory of Eukaryotic Molecular Biotechnology, Sfax University, Sfax, Tunisia.
Tuft cells function during intestinal tumorigenesis

Sixty years ago, ultrastructural observations of the gastro-intestinal tract allowed the morphological identification of tuft cells. Until their recent characterization by our group, tuft cells have been shown to express cellular markers such as DoubleCortin-Like Kinase 1 (Dclk1), which was initially described as putative quiescent stem cells. Interestingly, several reports also identified Dclk1+ve cells in early adenomatous intestinal lesions, sharing with tuft cells from the healthy tissue, all the known tuft cells markers. Importantly these tumoral « tuft-like cells » have been described in mouse and human lesions.

This project will focus on the intestinal tuft cells capacities to promoting initiation of tumorigenesis and sustain tumor growth. We will investigate:

1) Characterization of tuft cells in the healthy tissue. To assess the long-lived or short-lived status of these cells, we used a [Dclk1-CreERT2; R26mT/mG] mouse strain to perform lineage tracing. To complete this characterization, BrdU incorporation experiment confirmed that Dclk1+ve cells are short-lived ones, with an estimated turnover of less than two weeks.

2) Dclk1+ve cells status (Tumor Initiating Cells vs Tumor Propagating Cells). To determine if Dclk1+ve cells are TIC, we used a mouse model related with intestinal tumorigenesis devoid of tuft cells [ApcΔ14/WT; Pou2f3KO/KO]. We have showed that if tumorigenesis occurs without tuft cells, the number of intestinal lesions is significantly decreased. As complementary experiment, Pou2f3 deficient mice will be challenged with the well-established AOM-DSS treatment, which induce carcinogenesis within the large intestine, thus being more relevant for CRC.

To investigate how tuft cells could impact tumorigenesis, we will perform transcriptomic analyses on ApcΔ14/WT and ApcΔ14/WT; Pou2f3KO/KO derived-tumors. As we recently shown that tuft cells are able to activate enteric immune system, we will explore the hypothesis in which cancer tuft cells could induce the development of a pro-tumoral microenvironment.

Emmanuelle SIDOT, Salima SOUAHLI, Pierre CESSES, François GERBE and Philippe JAY
Gene expression is tightly controlled to ensure a wide variety of cell types and functions. The development of diseases, particularly cancers, is invariably related to deregulations of these controls. Our project aims to model the link between RNA expression and DNA features in regulatory regions (typically, presence/absence of transcription factor motifs [1] in promoters).

Several studies have shown that penalized linear regression (LASSO) is suitable for this problem, that requires selecting variables in high dimensional data [2]. Using similar approach, we were able to model gene expression in several cancers. Further investigations showed that the inferred model is not equally efficient for all genes. More precisely, the model only fits a certain class of genes with specific DNA features. This is likely due to the lack of suitable predictive variables for all other genes.

Our perspective is now to extend this model with other types of genomic variables, and to design a clustering approach to identify coregulated genes and train a specific regression model for each cluster.


May TAHA$^{1,2,3}$, Chloé BESSIERE$^{1,3}$, Florent PETITPREZ$^{1,3}$, Charles LECCELLIER$^{1,3}$, Laurent BREHELIN$^{3,4}$, Sophie LEBRE$^{2,3}$. $^{1}$IGMM $^{2}$IMAG $^{3}$IBC $^{4}$LIRMM
Oxytocin and vasopressin pace lateral septum electrical activity: consequences on brain rhythms and social memory

The mechanisms underlying oxytocin (OT) and vasopressin (VP) modulation of social behavior remain unclear. OT and VP receptors are expressed in the lateral septum (LS), a brain area involved in the regulation of social behavior and hippocampal theta oscillations. We therefore tested whether OT and VP in the LS have an impact on neuronal activity, electrographic and behavioral read-outs.

Effect of bath applied TGOT (specific OT receptor agonist) and VP on action potential frequency was quantified on acute brain slices. In vivo, cortical electroencephalograms paired with video were used to assess the effect of LS injections of OT/VP and their antagonists on awake mice. Social memory testing was also performed in these conditions after LS injection of OT/VP receptors antagonists.

Electrophysiological experiments defined 3 neuronal categories in the LS:
- Bursting neurons with AP frequency increased by VP only (50%)
- Bursting neurons with AP frequency increased by TGOT only (25%)
- Tonic neurons with AP frequency decreased by both peptides (25%)

Strikingly, TGOT and VP actions result from a modulation of inter-burst intervals (1.5-5s range) rather than intra-burst frequency (3-5Hz range). Furthermore, these neurons receive synaptic information whose frequency and patterning is also modulated by TGOT and VP (in a TTX dependent way). This indicates that LS neurons are involved in a local network paced by OT and VP.

In vivo experiments indicate that VP and TGOT change cortical EEG by decreasing power of frequencies under 6Hz and increasing others. Social memory also seems dependent on OT/VP receptor activation and we now try to figure out the link with EEG modification due to social encounter.

These results suggest an important role of septal OT and VP in the regulation of neuronal but also electroencephalographic activity in wild type mice with possible consequences on social memory.

Borie AM., Boussadia B., Gillon G., Augusto E., Marchi N., Desarmenien MG. Institut de Génomique Fonctionnelle UMR 5203 U1190 UM
N°10 (with Flash Talk) – Malik BOUASSE

Functional expression of the NALCN ion channel in a neuronal cell line

The membrane potential of neurons is determined by the intra- and extracellular ion concentrations and their membrane permeability. In this context, the NALCN ion channel is a G protein-coupled receptor-activated ion channel that conducts a depolarizing TTX- and Cs+-resistant Na+ leak current in neurons. It is involved in the regulation of neuronal electrical activity by hormones and neurotransmitters. In humans, both recessive and dominant mutations of NALCN were recently described in complex neurological disorders such as Infantile Neuroaxonal Dystrophy (INAD) and Type 2A Distal Arthrogryposis (CLIFHADD). These disorders share common symptoms such as ataxia, epileptic seizures, hypotonia, cognitive delay and developmental retardation. However, functional consequences of NALCN-related mutations found in diseases are not known mainly because of the lack of a reliable cellular model to achieve electrophysiological recordings. In the present study, we report that the neuronal NG108-15 cell line express the NALCN’s ancillary subunits Unc79 and Unc80. Thus, we transfected these cells with NALCN (wild-type or mutated), and its subunit NLF-1. Patch-clamp recordings revealed that dominant mutations found in CLIFHADD resulted in the expression of a strong and robust sodium inward leak current compared to control conditions. Surprisingly, no significant current was observed with the wild-type channel. These results suggested that mutations found in CLIFHADD are gain-of-function ones and that other factors are involved in the activation of the wild-type channel. Altogether, our data indicated that the NG108-15 cell line is a reliable cellular model to study functional consequences of dominant mutations of NALCN. However, more work is now required in order to determine the best conditions to observe a current with the wild-type NALCN channel.

Malik BOUASSE, Isabelle BIDAUD, Nathalie GUERINEAU, Philippe LORY, Arnaud MONTEIL
N°11 – Fabien RECH

Neural basis of motor control: study of the negative motor network with brain mapping

The primary motor cortex and the pyramidal tract have been considered for a long time as the main core of a single motor network. However, some neurological deficits can not be explained if the motor function is reduced to these primary motor structures. Indeed, studies showed that resection of tumors in the premotor areas can lead to permanent deficit in bimanual coordination and fine movements despite an efficient monitoring of the primary motor structures. We hypothesized that these deficits were observed because others motors networks were not monitored during surgery. Classically, the motor monitoring was performed by looking for positive motor responses, (i.e. muscle contraction) after electrostimulations during awake surgery or general anaesthesia. Cortical negative motor responses (NMR) are defined by a complete cessation of movement without loss of tonus and consciousness. They have been well described but their functional significance was not understood. Recent studies showed that it was possible to elicit NMR at a subcortical level for the upper and lower limb and the face. We described also subcortical NMR for bimanual movement and that the preservation of the corresponding sites prevented the occurrence of a permanent deficit. We assumed that there is a motor control network which might modulate the primary motor network and which is responsible of high skills such as bimanual coordination. Our goal is to explore this negative motor network thanks to direct electrostimulations. We described a somatotopic distribution of the NMR at a subcortical level, and added new datas concerning NMR of eyes movement. Actually we work on a probabilist cartography of cortical NMR to better understand their cortical origin. Once the cortico-subcortical distribution of the NMR in the motors areas will be performed, it will be possible to explore the connectivity with other brain regions by combining electrostimulations and neuroimagery (fMRI).

Fabien Rech\textsuperscript{1,2}, Guillaume Herbet\textsuperscript{2,3}, Sylvie Moritz-Gasser\textsuperscript{2,3}, Hugues Duffau\textsuperscript{2,3}. \textsuperscript{1}Department of Neurosurgery CHU Nancy. \textsuperscript{2}Team "Plasticity of Central Nervous System, Stem Cell and Glial Tumors", INSERM U1051, Institut for Neurosciences of Montpellier. \textsuperscript{3}Department of neurosurgery Hopital Gui de Chauliac, Montpellier.
Pesticides are organic or synthetic chemicals, used to preserve agricultural areas to improve yields but also for the preservation of food products. France is one of the largest users of pesticides and their massive utilization causes environmental pollution and food contamination that can lead to health problems. Epidemiological studies in humans have shown that two pesticides, rotenone and paraquat, could influence the risk of developing Parkinson's disease in a population exposed to heavy and/or frequent doses of these pesticides. Recently, Richardson and al., have shown that in some patients with Alzheimer's disease, the amount of DDE (DDT metabolite) in plasma is 3.8 times higher than in healthy people. In the laboratory, new molecules that interact with prion protein and promote the formation of PrPSc oligomers were identified. One of these compounds, A6, is a natural herbicide belonging to the family of α-terthienyls. This organic herbicide is synthesized by plants, as marigolds, and its toxicity is due to the formation of free radicals. We studied the effect of the A6 compound in a mouse model of prion disease. This molecule was tested at several concentrations: 5, 10 and 20 mg/kg. Our results show that at 5 mg/kg, the treatment accelerates the onset of pathology, correlated with an increase of amyloid deposits in the brain, as well as astrocytic gliosis and spongiosis. On the contrary, for higher doses, at 10 and 20 mg/kg, the treatment decreases the histo-pathological signs of prion disease. These results suggest that, depending on the concentration of the herbicide used, the aggregation process of the prion protein can be modulated.

N°13 – Camille PUJOL  
**GPRIN1, a new 5-HT6 receptor partner that regulates receptor-operated Gs signaling and neurite growth**

The serotonin 5-HT6 receptor (5-HT6R) is a Gs-coupled receptor expressed early during brain development predominantly in central nervous system regions linked to cognition. 5-HT6R antagonists are promising targets to treat cognitive impairments of schizophrenia, a well-known neuro-developmental psychiatric disorder. Thus, 5-HT6 receptor also plays crucial roles in both the correct migration and positioning of neurons, neuronal differentiation and neurite growth. These effects are agonist-independent and require the activity of Cdk5 associated with the receptor. Using an affinity-purification coupled mass spectrometry (AP-MS) proteomics strategy, we found that the receptor C-terminus also recruits G protein regulated inducer of neurite outgrowth 1 (GPRIN1), a Cdk5 substrate known to promote neurite outgrowth. GPRIN1 co-immunoprecipitation in NG108-15 cells, decreased with a mutated receptor unable to couple with G proteins, suggesting a G protein-dependent association. This interaction also depends on Cdk5 activity and is dynamically modulated by receptor phosphorylation at Ser350 by Cdk5. Co-expressing GPRIN1 with 5-HT6 receptor in NG108-15 cells increases the length of primary neurites and the number of secondary neurites, as shown by Scholl analysis. Moreover, GPRIN1 co-expression strongly increases receptor constitutive activity at Gs signalling and decreases the potency of inverse agonists, suggesting that GPRIN1 association stabilizes an active state of the receptor. Collectively these data identify GPRIN1 as a novel partner of 5-HT6 receptor that enhances receptor constitutive activity and its promoting effects on neurite growth. They suggest that 5-HT6 receptor-operated neuronal differentiation depends on a complex sequence of events involving sequential and strongly interrelated associations of receptor with different interacting proteins.

_C. Pujol¹, M. Séveno¹, J. Bockaert¹, P. Marin¹, S. Chaumont-Dubel¹. ¹Institut de Génomique Fonctionnelle, Neuroscience, Montpellier Cedex 05, France._
Decoding of a new molecular mechanism in the regulation of early autophagic steps

The autophagic route is a major degradation pathway that sustains cellular homeostasis in basal condition, but also in response to stress. In this process, an autophagosome engulfs specific cytosolic components, matures and subsequently fuses to a lysosome to form the autophagolysosome, in which acid hydrolases degrade its content. While late steps of autophagosome maturation are relatively well studied, little is known about the early steps of autophagosome formation.

In this project, we investigated the role of gigaxonin in the regulation of the early steps of autophagy. Gigaxonin is an adaptor of an E3 ligase. This family of enzymes is responsible for the tagging of ubiquitin chains on specific proteins, leading to their degradation by the proteasome. Mutated in a fatale neurodegenerative disorder called Giant Axonal Neuropathy (GAN), gigaxonin has been shown, both in patients and cellular/animal models of the pathology, to bear crucial functions in sustaining neuronal survival and cytoskeletal organization, through its ubiquitinating activity.

To assess for the role of gigaxonin in the autophagy-dependant degradation, we analyzed cortical neurons from GAN+/+ and GAN+-/- 15.5 aged embryos. In particular, we evaluated the effect of gigaxonin depletion on autophagosome formation, autophagic flux, lysosome fusion and degradation. We revealed a defect in autophagosome formation in GAN+-/- neurons. Using complementary techniques, we showed that gigaxonin is essential for the functional turn-over of an essential ATG protein, through its E3 ligase activity.

Altogether, we identified a new molecular mechanism in the regulation of the early step of autophagy. Not only these findings present a significant advance in the comprehension of the fundamental regulation of autophagy, but they also contribute in the understanding of its dysfunction in neurodegenerative diseases, and generate a new target for therapeutic intervention in humans.

Scrivo, A. (Montpellier)\(^1\), Codogno, P. (Paris)\(^2\), Bomont, P. (Montpellier)\(^1\). \(^1\)Atip-Avenir Team, INM- INSERM U1051, Montpellier, France. \(^2\)INSERM U845, Necker Growth and Signaling Research Center, University Paris Descartes, Paris, France.
Generation of Alzheimer's disease (AD) genetic patients’ reprogrammed stem cells (iPS) as tools for the study of AD physiopathology

Amyloid precursor protein (APP) and Tau protein are two main molecular actors of neurodegenerative affections, which are of prime importance in Human Health (Alzheimer’s disease (AD)). Intensive research is ongoing to understand these proteins’ metabolism, action and implication in the pathological mechanism of these affections. They are the target of most therapeutic approaches and are used for biological diagnosis. In the present program, our objective is to investigate neuronal APP and Tau protein processing and metabolism using biochemical tools (single and multiplex immunodetection system (ELISA, Luminex®, MSD®)), innovative detection methods (mass spectrometry (MS)) and metabolic approach (incorporation of stable isotope labelled Leucine (6C13L)) in two complementary situations: in vivo in patients, and in vitro in cell culture. The goal is to get a comprehensive proteomic view (synthesis, cleavage, interaction..) based on the parallel analysis of patient isotope labelled samples (already available following perfusion of 6C13Leu and kinetics sampling of CSF) and samples generated in neuronal differentiated human embryonic stem cell and Induced pluripotent stem cells derived from AD-patients where pharmacological tools (secretase/kinase inhibitors) can be tested. The final goal is therefore to parallel the data in patients with those generated in differentiated iPS cells and control hESC cells. This project will offer the unique opportunity to combine state-to-the-art approaches to understand how the APP fragments and peptides are generated as well as the modifications of the Tau protein in normal and pathological situation.


1Institut de Médecine Régénératrice et de Biothérapie (IRMB), Montpellier, France
Charcot-Marie-Tooth (CMT) diseases are inherited diseases that affect peripheral nerves in humans. More than 70 gene mutations have been shown to cause CMT diseases. This neurologic disorder cause muscle weakness in the feet, ankles, legs and hands, an awkward way of walking (gait), highly arched or very flat feet and numbness in the feet, arms and hands. There is no cure for CMT diseases. One approach for a treatment is gene therapy. In this study, we intend to examine whether this approach is efficient for treating rat model of CMT1A, the most frequent type of CMT diseases. This model is characterized by a high expression of PMP22, a small protein that leads to demyelination when overexpressed. CMT1A rats will be injected with adenovirus viral vectors AAV9 shRNA PMP22. The efficiency of this therapy will be checked by assessing muscle strength (grip test), way of walking (catwalk) and the mobility (rotarod) of treated animals versus non-treated. The process of myelination and myelin maintenance in Schwann cells will be analyzed by biochemistry and electron microscopy. Biochemical tests include Western Blot for PMP22 protein expression in sciatic nerve, immunohistochemistry for PMP22 protein expression in myelinating Schwann cells and PCR for PMP22 mRNA expression. Reduction of PMP22 overexpression and hence inhibition of demyelination is expected. If the therapy is successful in rats, it could possibly be later on used in clinical trials.

Helene Hajjar¹, Jade Berthelot¹, Nicolas Tricaud¹. ¹INM
N°17 (with Flash Talk) – Nathalie NASR
The role of the mechanosensitive ionic channels during zebrafish heart regeneration and development

Cardiovascular disease represents the primary cause of death in the world. In human most cardiovascular disorders will lead to a destruction of the cardiac tissue which will be replaced by fibrosis leading to arrhythmia and reduced contractile function. The loss of cardiac tissue results in an increase in ventricular load. The heart responds to this increased load by inducing cardiomyocytes to undergo hypertrophy in order to maintain overall cardiac output. Ultimately this will result in pathological hypertrophy and heart failure. The increase in ventricular load which triggers the hypertrophic response can be potentially sensed by mechanosensors such as TREK1 and TRPC6. Unlike mammals, adult zebrafish can fully regenerate their heart after an extensive insult through dedifferentiation and proliferation of cardiomyocytes. We believe that in adult mammals cardiomyocyte proliferation has been blocked/inhibited resulting in pathological hypertrophy. Therefore it is likely that genes which respond to increased ventricular load in mammals and trigger pathological hypertrophy will trigger cardiomyocyte proliferation during heart regeneration in zebrafish. By identifying the mechanosensors (such as TREK1 and Trpc6) responsible for detecting changes in ventricular loading and triggering cardiomyocyte proliferation in zebrafish we will be able to identify the downstream effectors of these signaling pathways which may be blocked/inhibited in adult mammals, resulting in cardiomyocyte hypertrophy rather than cardiomyocyte proliferation and regeneration. Our goal is directly assay whether TREK1 or TRPC6 are required for successful heart regeneration in zebrafish. To achieve this we will adopt 2 approaches. Firstly, we will pharmacologically inhibit these channels during heart regeneration to determine whether this disrupts this process. Secondly, we will generate conditional transgenic zebrafish based on the Cre/Lox system to specifically express dominant negative forms of TREK1 and Trpc6 in cardiomyocytes during heart regeneration in order to determine whether these genes are involved in this process.

NASR Nathalie, MOHAOUMAATI Hamid, LAARIOUI Sihame, El Rhoddani Imane, JOPLING Chris
N°18 (with Flash Talk) – Matthieu RENAUD
A rat-tail model for bone regeneration and implant studies

Aim: EU directive for the protection of laboratory animals focuses on improving the number of animals. We are developing a rat-tail model in which vertebra architecture decreasing animal number for filling biomaterials and for dental implant models.

Materiel and methods: Adult Wistar rats (380 to 450 g) were selected. After, skin and muscles retractions on the dorsal side, the vertebrae were exposed. One defect is drilled in each caudal vertebra and four vertebrae are operated per animal.

Implantable material: defects of 3 mm of diameter were performed with a drill on 4 vertebrae. Two vertebrae were used like control site (empty of materials) and two other vertebrae were used for Bio-Oss® implantation for an healing period of one month and two months.

Implants: impacted-type of titanium implant in a drilling of 2.8 mm of diameter was used. In each rat, four vertebrae were used for titanium implant placement for an healing period od three months.

After different healing period vertebrae are analyzed by μCT in living rat. After sacrifice histological evaluation is performed.

Results: μCT and histology demonstrate that bone formation was absent in empty defect of 3x3 mm after one month and two months. When using different biomaterials we obtained a good retention of the material into the defect after one and two months. The implants were stable and osseointegration was revealed by histology.

Conclusion: The rat-tail vertebrae is an ideal model for bone tissue engineering and for implant experiments, decreasing in the same time the number of the animal.

Matthieu RENAUD, Msc, PhD student¹, Philippe BOUSQUET, MCU-PH, PhD¹, Frédérique CUNIN, PhD², Sylvie MONTAL, PU-PH, PhD¹, Frédéric CUISINIER, PU-PH, PhD¹. ¹EA4203 laboratory, faculty of dentistry, université montpellier. ²ICG, université montpellier.
Fluorescent protein tag interrupts complete switching of the bacterial flagellar motor

The bacterial flagellar motor (BFM) is the macromolecular complex which allows bacteria to swim in liquid media. This remarkably small, yet powerful biological rotary electric motor rotates up to 300 Hz (for e.coli) and switches the direction of rotation. The key components of the motor are the stators proteins (MotA and MoB) and the C-ring switching complex at the base of the rotor. It has been known that when MotB protein is fused to a fluorescent protein, the motor becomes functional but with reduced motility. As the fluorescent protein tag lies in the interface where the motor generates torque and switching, here, using single-molecule biophysics approach, we investigated the detailed aspects of the motors dynamics tagged by three fluorescent proteins (eGFP, Ypet, Dendra2). Single motors and single stator with Ypet-tagged stators produce the same amount of average torque as WT, despite their reduced chemotaxis ability. On the other hand, single motors and single stators with stators tagged by eGFP and Dendra2 produce 70% and 40% average torque with respect to WT. Interestingly, the fusion stator motors show significant differences in their switching abilities. When switching direction of rotation, the absolute value of the speed of WT motors does not change, whereas this symmetry of speed upon switching is not observed in the fusion stator motors; switching can be accompanied with a significant (~30%) decrease in absolute speed. Also, fusion stator motors spend longer periods of time of rotation in CW and CCW states than in WT, which reflects a lower switching frequency. Considering the proximity of the fluorescent tags to the MotB-FliG interface, the impaired torque production and the ability to switch could be due to frustrated conformational changes of the stators and of the switch complex.

Minyoung Heo, Francesco Pedaci
TGFBI: a potential regulator of cartilage homeostasis deregulated in osteoarthritis

As the most common form of joint disease, Osteoarthritis (OA) represents a significant public health problem that currently lacks curative treatment. In this context, cellular therapy appears to be an interesting approach, particularly using Mesenchymal Stem Cells (MSCs) which have demonstrated a therapeutic role in OA. In order to identify new targets involved in the therapeutic effect of MSCs and cartilage homeostasis, we analyzed the secretome of MSCs by MS/MS. We focused our attention on Transforming Growth Factor β-Induced protein (TGFBI), a poorly studied member of the TGFβ family.

We first confirmed the expression of TGFBI mRNA in both human and murine primary MSCs. In the mouse, the transcript was preferentially expressed in cartilage although detected in bone and bone marrow. We then developed in vitro models to investigate the potential role of TGFBI in OA cartilage. We used primary murine chondrocytes and mouse femoral head explants and added IL1-β in the cultures to mimic OA environment. In both models, IL1-β-treated samples exhibited an OA-like phenotype characterized by decreased expression of chondrocyte markers and increased expression of matrix degrading enzymes and inflammatory factors. TGFBI was significantly downregulated in IL1-β-treated chondrocytes suggesting a down-regulation of TGFBI in OA. However TGFBI was not down-regulated in IL1-β-treated explants possibly because an increased expression in the bone compartment of the explant could normalize the overall secretion. Finally, we explored the therapeutic potential of murine MSCs on IL1-β-treated chondrocytes either in co-culture or by adding conditioned medium. In both cases, we observed a partial reversion of the OA-like chondrocyte phenotype and this was associated with the upregulation of TGFBI. Our results suggest that TGFBI may be a key regulator of chondrocyte biology but further investigations are needed to understand the role of this factor in cartilage integrity and the therapeutic effect of MSCs.

Ruiz M\textsuperscript{1,2}, Maumus M\textsuperscript{1,2}, Toupet K\textsuperscript{1,2}, Jorgensen C\textsuperscript{1,2,3}, Noël D\textsuperscript{1,2,3}. \textsuperscript{1}Inserm U1183. \textsuperscript{2}Université Montpellier. \textsuperscript{3}CHU Montpellier, Unité Clinique d'Immuno-Rhumatologie, Montpellier, France. daniele.noel@inserm.fr
Role of the newly discovered XPR1 phosphate exporter in phosphate regulation and calcification

Phosphate is a key body mineral that plays a major role in nucleic acid and membrane synthesis, bone and tooth mineralization, energy production (ATP), and kinase/phosphatase regulations. Phosphate transport alterations may lead to perturbed phosphate homeostasis with severe clinical consequences. Our lab has previously identified XPR1, the retroviral receptor for xenotropic and polytropic murine leukemia viruses, as the first metazoan phosphate exporter. XPR1 thus fits in the list of solute carriers (SLC) that are used as receptors by gamma- and deltaretroviruses. Notably, PiT1/SLC20A1 and PiT2/SLC20A2, which also function as gammaretrovirus receptors, are sodium-dependent phosphate transporters that play a major role in cellular phosphate homeostasis.

PiT2 mutations have been identified in 40% of patients with primary familial brain calcification (PFBC), a rare neurodegenerative disorder characterized by calcium phosphate deposition in the basal ganglia of the brain. More recently, we identified mutations in XPR1 that are involved in PFBC, and demonstrated that such deleterious mutations could alter XPR1 expression at the cell surface and/or phosphate export in patient peripheral blood cells. The general aim of my work is to elucidate the role of XPR1 in phosphate homeostasis and metabolism at the cellular level.

All XPR1 deleterious mutations found in PFBC are contained within an amino-terminal cytoplasmic domain known as SPX, a domain shared by many plant and yeast proteins involved at different levels in phosphate metabolism. We have shown that siRNA-mediated down-modulation of XPR1 phosphate export function can be complemented with wt XPR1, whereas PFBC XPR1 mutants did not reestablish phosphate efflux. Since these results unveil the role of XPR1 SPX as a regulator of phosphate transport, we are currently seeking for XPR1 cellular partners that regulate XPR1-mediated phosphate transport. We are also assessing potential interactions between XPR1 and PiT2 in phosphate regulations.
Polycomb group proteins (PcG) form two main complexes, PRC2 and PRC1 that collaborate to establish stable long term silencing of HOX genes. Mutations affecting either PRC2 or PRC1 components in drosophila embryos lead to similar phenotypes i.e major developmental defects and lethality associated with HOX genes derepression.

In this work, we analyze the dynamics of PcG proteins targeting during fly development. Comparative analysis of ChIP-seq experiments has been performed against PRC1 components and H3K27me3 (deposited by PRC2) in embryonic versus larval eye-antennal imaginal discs. This work shows that PRC1 and H3K27me3 overlap in embryos and are kept during larval stages as expected. However, we identified a large set of genes, we named “neo-PRC1”, that acquires PRC1 in the absence of H3K27me3 only during larval stages. These target genes massively outnumber canonical targets and are, surprisingly, actively transcribed. Furthermore, PRC1 mutations trigger neoplastic tumors and massive overgrowth while PRC2 mutations result in small disc phenotype. PRC1-dependent tumorigenesis is characterized by Notch transcriptional up-regulation, impaired differentiation, polarity defects and ectopic proliferation. Moreover, neo-PRC1 target genes are regulators of cellular signalling, polarity and proliferation. Finally, neo-PRC1 target genes are specifically deregulated in PRC1 mutants while canonical targets are derepressed at comparable levels in both PRC1 and PRC2 mutants.

Remarkably, in human ESCs, PRC1 and PRC2 components colocalize, a situation that resembles the situation of fly embryogenesis, whereas in differentiated fibroblasts the recruitment of PRC1 components may involve H3K27me3-independent mechanisms.

Altogether, these results show that PRC1 components act as neoplastic tumor suppressors independently of PRC2 function and suggest that the redeployment of PRC1 components during development is evolutionary conserved.

Vincent Louibiere\textsuperscript{1,*}, Anna Delest\textsuperscript{1,*}, Aubin Thomas\textsuperscript{1}, Boyan Bonev\textsuperscript{1}, Bernd Schuettengruber\textsuperscript{1}, Anne-Marie Martinez\textsuperscript{1,2,°}, and Giacomo Cavalli\textsuperscript{1,°}. \textsuperscript{1}Institute of Human Genetics, UPR1142 CNRS, 141 rue de la Cardonille, 34396, Montpellier Cedex5, France. \textsuperscript{2}Université de Montpellier, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France. *Contributed equally to the work. °Corresponding authors.
Glutaminolysis and FAO regulate the relative commitment of HSCs to myeloid and erythroid lineage fates

The self renewal capacity of hematopoietic stem cells (HSCs) is controlled by the cells’ metabolic state but the possibility that nutrient entry and metabolism contribute to the differential commitment of an HSC to a distinct lineage fate was not considered until very recently. We previously found that inhibiting ASCT2 glutamine transporter function or glutaminolysis diverts erythropoietin-signaled human HSCs to a myeloid fate. Mechanistically, erythroid specification requires glutamine-dependent de novo nucleotide biosynthesis (Oburoglu et al., CSC, 2014). As glutaminolysis induces mTOR activity, we have now investigated the role of mTOR-mediated signaling cascades in regulating the commitment “choice” to an erythroid versus myeloid lineage fate. Surprisingly, different mTOR inhibitors had disparate effects on erythroid-myeloid lineage choice in the presence of recombinant erythropoietin (rEPO). We found that this was due to distinct changes in fatty acid oxidation (FAO); enhanced FAO increased erythroid differentiation whereas blocking this pathway skewed rEpo-stimulated HSCs to a myeloid cell specification. Thus, amino acid metabolism and fatty acid oxidation coordinately regulate the relative differentiation of HSCs to myeloid and erythroid lineages.

Romano M*, Oburoglu L*, Taylor N# and Kinet S#. UMR5535 Institut de Génétique Moléculaire de Montpellier, 1919 Route de Mende, 34293 Montpellier, Cedex 5, France; *#equal contribution
N°24 – Elena LO FURNO
Exploring the molecular grounds of genomic instability in early embryos

Early embryos show signs of genomic instability of unclear origin. During the rapid cell divisions of early embryogenesis canonical genome surveillance mechanisms, such as DNA damage checkpoints are inefficient and may contribute to genomic instability. In early Xenopus laevis embryos the DNA damage checkpoint is largely suppressed by constitutive recruitment of translesion (TLS) polymerases onto replication fork; thus preventing single-stranded DNA accumulation, a critical substrate for checkpoint activation (Kermi et al., Dev Cell 2015). These observations imply the possible occurrence of elevated mutagenesis during early embryogenesis, due to the error-prone nature of TLS.

My thesis project is aimed at understanding the contribution of TLS in mutagenesis during early embryogenesis and to unravel the origin of genomic instability of early embryos.

Firstly, we provide evidence for constitutive TLS activation during early embryo development also in Drosophila melanogaster. Then, in order to assess the impact of TLS in mutagenesis during Drosophila early embryogenesis, we generated TLS homozygous or heterozygous maternally-deficient flies that expressed TLS only after the Mid-Blastula Transition (dpolη mutants) and monitored the development of these flies from embryo to adult stage. The results obtained showed that homozygous dPolη maternally-deprived embryos displayed higher mortality and reduced hatching rate in comparison to heterozygous. Further, Whole-Genome Next Generation Sequencing (NGS) analysis showed a reduced rate of mutagenesis in these mutant flies. Intriguingly a hortologue of a TLS master regulator, the Rad18 ubiquitin ligase (E3) in Drosophila, has not yet been found. Finally, we are investigating the nature of genomic instability in embryonic stem cells and induced pluripotent stem cells and a possible contribution of the TLS. Altogether, these observations suggest that TLS may be one unexpected source of genomic instability in early embryos that may contribute to the genetic polymorphism in adult cells. Other sources may exist though these remain to be identified.

Elena LO FURNO, *Isabelle BUSSEAU, **Cima SAGHIRA, **Stephan ZUCHNER & Domenico MAIORANO. Laboratoire “Surveillance et Stabilité du Génome” *Laboratoire “ Impact systémique des petits ARN régulateurs” ** Hussman Institute for Human Genomics, University of Miami (USA) Institut de Génétique Humaine (IGH) CNRS UPR1142. Université de Montpellier, Montpellier, France
N°25 – Nabiya BOUBACAR ALI
Characterization of the signaling pathway of an enzyme of epigenetic path H4-K20me

Chromatin organization is modulated by a large set of different histone modifications induced by specific enzymes, which play key roles in all nuclear processes. In the laboratory, we are interested in understanding the functions of the lysine methyltransferase PR-Set7, responsible for the methylation of histone H4 at lysine 20 (H4K20me).

PR-Set7 activity is essential during development of multicellular organisms. Mouse embryos devoid of PR-Set7 display defects in DNA replication and chromatin structure and fail to develop beyond the four-cell stage, which has suggested a key role in this enzyme in the maintenance of genome integrity during the cell cycle. To characterize further the role played by PR-Set7 during development, we have generated tools to study the ortholog of PR-Set7 in Drosophila (dPR-Set7). We recently observed in flies that dPR-Set7-dependent cell proliferation might depend on transcriptional regulation of key regulatory genes for the cell cycle. Interestingly, transcriptional regulation mediated by PR-Set7 clearly necessitates its functional enzymatic SET domain but does not require H4K20me, suggesting the requirement of non-histone PR-set7 substrates.

My thesis project will intend to dissect a strong genetic interaction observed between dPR-Set7 and the chromatin remodeling ATPase protein ISWI. ISWI is a regulator of transcriptional mechanisms that contains a basic patch identical to H4 N-terminal tail, suggesting that it could be a substrate of dPR-Set7. Using molecular, cellular and genetic approaches, we would like to determine (1) if PR-Set7 and ISWI belong to similar protein complexes, (2) if ISWI can participate in the transcriptional regulation of PR-Set7 target genes, (3) if ISWI could be a substrate of dPR-Set7 and/or if PR-Set7 can modulate ISWI remodeling activity. These experiments that would be mainly done in Drosophila would serve as a basis to clarify the impact of PR-Set7 in the regulation of transcription in mammals, a function that could be also independent of H4K20me.

Directeur de thèse : ERIC JULIEN
Co-Encadrante : Charlotte GRIMAUD
Branch-chain amino acids (BCAA) are potential determinants of insulin resistance either by variation of plasma levels or dietary intake. To understand their implication in obesity, a new screening strategy of candidate SNPs (single nucleotide polymorphism) was applied but different from previous approaches using leader SNPs. We considered 52 genes in BCAA metabolism and related pathways, retrieved SNPs from dbSNP and complete with 1000 Genome project (1KGP) and proxies databases. From 214,092 collected SNPs we had available 907 SNPs on our Axiom MEDISCOPE gene chip, which satisfied QC. To identify hundreds of potential influential SNPs with OR > 2.5 and MAF > 0.4 at a power of 0.8, we included 324 subjects for genotyping (80 obese patients and 244 controls). Obese patients display simple or morbid obesity at age of 38 ± 0.9 y (mean ± SEM), BMI of 39 ± 0.43 (22.98 ± 0.39 in lean) and with HOMA values of 3.3 ± 0.2 (1.9 ± 0.2 in controls). All obese were also investigated by IVGTT. They were homogenous compared to CEU population in PCA after pruning and filtering SNPs with MAF > 0.01. In logistic regression only rs8044145 of ACSF3 (acyl-CoA-synthetase) gene was associated with significant P < 1.5x10^-5, OR of 2.72, 95%CI [1.57- 4.71] and sustained by Bonferroni correction. Strong correlation was also observed with BMI and HOMA index. Not sustained by Bonferroni correction there were some other associated SNPs in genes: rs12700830 (HIBADH), rs864745 (JAZF1), rs1872645 (BCAT1), rs12914710 (IVD), rs114612460 (GCKR), rs3805850 (BCKDHB), rs77048944 (AASC), rs57802801 (ABAT). SNP rs864745 of JAZF1 gene showed good correlation with BCAA plasma levels (P < 0.03). These data indicate a potent role of genes from BCAA metabolism in insulin resistance and reinforce our novel approach in gene SNPping in complex conditions.

Sara Haydar¹, Yannick Cogne¹, Jean Christophe Hadi¹, Landy Randrianarisoa¹, Jean Frederic Brun², Eric Renard², Jean Marie Robine³, Corinne Lautier¹, Jean Paul Cristol² and Florin Grigorescu¹. ¹UMR-204, NUTRIPASS (IRD/UM/SUPAGRO), Montpellier. ²CHU Montpellier. ³Montpellier University
Autophagy is a potent anti-HIV-1 mechanism. It is triggered in CD4 T cells by the viral envelope (Env) upon HIV-1 entry. In bystander CD4 T cells, autophagy leads to apoptosis. In productively infected CD4 T cells, autophagy is inhibited, preventing thus HIV-1 virophagy and Env-mediated apoptosis. Here we demonstrate that HIV-1 Vpr contributes also to the inhibition of autophagy. Indeed, both ectopic expression of Vpr and Vpr incorporated into the virions decrease the number of autophagosomes in CD4 T cells when autophagy is induced by an inhibitor of mTOR and Env, respectively. To define the mechanism by which HIV-1 Vpr inhibits autophagy, we performed GST pull-down experiments and identified that Vpr interacts with BNIP3, a pro-autophagic protein. Importantly, BNIP3 expression level is increased in CD4 T cells upon Env contact, suggesting that BNIP3 could be responsible for the Env-mediated induction of autophagy. Furthermore, Vpr co-localizes with BNIP3 and viral incorporated Vpr decreases BNIP3 levels after 8 hours of infection. In conclusion, we demonstrate that Vpr controls autophagy during the early phase of infection. The complete understanding of the mechanisms by which HIV-1 inhibits autophagy should lead to the rising of new molecular strategies to fight against this virus.

Jamal Alfaisal, Coralie Daussy, Véronique Robert-Hebmann, Lucile Expert, Martine Biard-Piechaczyk. Centre d’études d’agents Pathogènes et Biotechnologies pour la Santé (CPBS-CNRS), Université de Montpellier Montpellier - France
Infection of human placental cells by Brucella strains causing abortion in baboons

Brucella are intracellular bacteria causing the zoonotic disease Brucellosis. Among the different species of Brucella, some of them are described as pathogenic for humans. In female animals, infection is characterized by abortion and sterility. Infected pregnant women may have higher risks of obstetric complications, but this is not well documented. Some zoonotic Brucella species can replicate within human trophoblasts, specialized cells with immune and hormonal functions essential for placental development. Infection of trophoblasts by Brucella may alter these functions.

We are studying the infection of human trophoblasts by strains of Brucella (B. papionis) that caused abortion in non-human primates. We first evaluated the intracellular behavior of these strains in immortalized human cytotrophoblasts (CTBs) and extravillous trophoblasts (EVTs). CTBs are placental stem cells that differentiate into syncytiotrophoblasts, forming an epithelium in contact with the maternal blood, or EVTs, with phagocytic properties constituting the first immune barrier in the placenta. EVTs participate in the anchorage of the embryo and in the remodeling of uterine blood vessels to allow proper blood supply in the placenta. We found that B. papionis invade human CTBs and EVTs, but are only able to replicate efficiently within CTBs, suggesting that EVTs can restrict the intracellular growth of B. papionis. Observation of the subcellular localization of the bacteria using a live/dead reporter system showed that, if B. papionis replication is controlled in EVTs, the bacteria can survive in these cells. The two types of intracellular behaviors of B. papionis in trophoblasts could affect their functions or cause an inflammation in the placenta, explaining the pathogenicity of these strains during pregnancy. We will thus evaluate the consequences of B. papionis infection on the functions of CTBs and EVTs and production of cytokines by these cells. With this, we hope to understand the mechanisms of Brucella pathogenicity during pregnancy.

García-Méndez KB1, Gorvel JP2, Arce-Gorvel V2, O’Callaghan D1, Keriel A1. 1Inserm U1047, Nîmes France, Université de Montpellier. 2Centre d’Immunologie de Marseille-Luminy, Aix-Marseille Université UM2.
N°29 (with Flash Talk) – Tran THI THU PHUONG
The indirect impact of immune-complexed adenovirus on dendritic cells

Human adenovirus type 5 (HAdV-C5), is nonenveloped double-stranded DNA virus with 36 kb genome. It is well characterized as a vector for vaccination, gene transfer and cancer treatment (oncolytic vectors). However, the ubiquitous pre-existing immunity to HAdV-C5 is a complicating issue for use of vaccines and gene transfer vector. We recently showed that immune-complexed HAdV-C5 (IC-Ad) induces functional maturation, inflammatory responses, and a pro-inflammatory death of dendritic cells (DCs) called pyroptosis. We therefore asked “Does the pyroptotic environment created by IC-Ad-stimulated DCs impact the subsequent immune response?” Of note, recruitment of monocytes is essential for effective control and clearance of viruses, but recruited may contribute to disease pathogenesis. We therefore characterized i) indirect-stimulated DCs (indir-DCs) and their roles in the immune response; ii) the relationship between direct-DC (dir-DCs) stimulation and monocyte migration; and iii) the properties and phenotypes of indir-DCs and direct-activated DCs (dir-DCs) to determine factors influence their maturation and monocyte migration. In this system we used biochemical and cellular assays to evaluate the secretome of indir-DCs and monocyte migration in a transwell system. We found that indir-DCs mature after exposure to a dir-DCs environment and, surprisingly, the dir-DC environment decreases monocyte migration, which was due to pyroptosis. These data suggest that the environment created by dir-DCs will impact vaccine, gene transfer, and oncolytic virus therapy.

Thi Thu Phuong TRAN, Karsten EICHHOLZ, Franck MENNECHET & Eric J. KREMER
Development of a multi-epitope peptide vaccine against human leishmaniasis

Leishmaniasis is a vector-borne neglected tropical disease endemic in 98 countries globally, including Europe, where zoonotic reservoirs are crucial for stable transmission. Sixteen Leishmania species are capable of establishing intracellular infection within macrophages, causing different clinical presentations - cutaneous, mucocutaneous and visceral leishmaniasis. Natural protection against infection is characterized by a dominant Th1 cellular immune response, with production of IFN-γ, IL-2 and TNF-α by CD4+ T cells, ultimately leading to macrophage activation and parasite killing, whereas Th2 responses are associated with infection progression.

We aim to develop an epitope-based peptide vaccine from proteins present in the Leishmania secretome, a major player in host/pathogen interaction. Total excreted/secreted antigens of 6 different pathogenic species were analysed by Mass-Spectrometry enabling the identification of both novel and previously known vaccine candidates. We propose an innovative reverse vaccinology approach based on the secretome proteomic data, wherein we will perform in silico analysis of selected proteins to predict and select the best CTL- and Th1-inducing epitopes, subsequently synthesized as peptides. The proposed peptide candidates will be tested experimentally with samples from naive, asymptomatic and healed individuals, providing the valuable opportunity to directly infer on their ability to mimic protective immune responses and elicit a strong and long-lasting immunity against leishmaniasis.

Pissarra JS1, Holzmuller P2, Lemesre JL1. 1UMR IRD-CIRAD INTERTRYP, Montpellier FR. 2UMR CIRAD-INRA CMAEE, Montpellier FR.
Analysing the role of secretion systems in the physiology and virulence of Brucella

Brucellosis is a widespread zoonotic disease caused by members of genus Brucella. The human disease represents an important cause of morbidity worldwide whereas animal brucellosis is associated with serious economical losses caused mainly by abortion and infertility in ruminants. Understanding the biology of Brucella is an essential step in the development of control measures.

Our laboratory discovered and is studying the VirB type IV secretion system (T4SS), used by Brucella to translocate effector proteins that modulate host cell biology and allow Brucella to survive and multiply within host cells. We also believe that Brucella can secrete effectors by other secretion system: the twin arginine pathway (Tat). The Tat pathway is present in many, but not all, bacteria and is used to export folded proteins from the cytosol to the bacteria envelope or the extracellular environment. The aim of this study is to investigate the role of the Brucella Tat system, to identify substrates exported by the Tat pathway to define the role that this pathway plays in the physiology and virulence of Brucella. To accomplish this, we are creating mutants with deletions of the operon encoding the Tat system and will assess their virulence in cell culture models. Proteins exported by the Tat system will be identified with a combination of in silico, genetic and biochemical screens and their roles in Brucella physiology and virulence analysed.

Elia Riquelme¹, David O'Callaghan¹. ¹Inserm U1047, Nîmes, France, Université de Montpellier.
**N°32 – Julie MIELLE**

**Function of IL-10 producing B cells in healthy subjects and patients with rheumatoid arthritis and Sjögren's syndrome**

Background: Rheumatoid arthritis (RA) and Sjogren’s syndrome (SjS) are the most frequent auto immune diseases in France. In RA, treatments are only suspensive whereas in SjS, therapies are lacking. New therapeutic targets are thus needed. B cells are crucial players in autoimmunity. However, B cells can also have regulatory functions through the production of the anti-inflammatory Interleukin (IL) 10. We recently showed that RA patients have less B cells able to produce IL-10 (B10) than healthy controls. More interestingly, the number of B10 was negatively correlated with the disease activity and the levels of auto antibodies. A preliminary work in the lab showed a lack of B10 in SjS. Taken together, these results suggest that B10 can play an important role in auto-immune disease. To date, B10 cells in human are not well characterized. We therefore aimed to study the functions of B10, on the different immune cells involved in RA and SjS.

Methods: Using isolation kits and cytometry, we sorted B10 cells and evaluated their capacity to differentiate T cells into a regulatory (Treg) or an inflammatory phenotype (Th1, T producing TNF).

Results: Our first experiments showed that in controls, B10 cells increased Treg (p=0.047, n=6) and decreased Th1 cells (p= 0.03 n=7). No effect on T-TNF was observed. In RA, the increase of Tregs by B10 seems conserved (n=4).

Conclusion: Knowing that B10 are still functional in RA, it could be interesting to find a way to increase the number of B10 cells in patients. Metabolism pathways are involved in T cell differentiation. To date, B10 metabolism was not studied. We therefore aim to study the metabolism of B10 cells, which could be an interesting strategy to increase their differentiation.

Mielle J1,2, Nutz A3, Audo R1,2,3, P Guillepain3, Combe B1,3, Hahne M1,2, Morel J1,3, Daien C1,2,3. 1Montpellier University, France. 2Institut de Génétique Moléculaire de Montpellier, CNRS, UMR5535, Montpellier, France. 3Department of Rheumatology, Montpellier University and Lapeyronie Teaching Hospital Montpellier France.
N°33 – Laure PAPIN
Characterization of early events of HIV-1 entry into dendritic cells and their impact on viral transmission

Cell-to-cell transmission facilitates propagation of pathogens including HIV-1, human T cell leukemia virus type 1 (HTLV-1), as well as other viruses and pathogens. Cell-to-cell transmission of HIV-1 is an obstacle to antiretroviral therapy, vaccine development and promotes HIV-1 immune escape. Special junctions formed between cells, in particular cells of the immune system, termed Virological Synapses (VS) mediate cell-to-cell transmission of pathogens. It has been demonstrated that some Dendritic Cell (DC) subsets could be involved in the very early events of HIV-1 infection and spread, which appear to rely on specific cellular receptors, such as the C-type lectin receptor DC-SIGN, engaged during viral entry.

We have recently reported a surprising role for DC-SIGN in targeting incoming virions toward antiviral autophagy (virophagy). Although timely counteracted by the virus in DC, virophagy appears to restrict HIV-1 infection of DC and viral transfer toward target CD4+ T cells while regulating DC-mediated innate and adaptive immune responses.

We hypothesize that the engaged HIV-1 receptors can initiate the antiviral response by targeting HIV-1 toward lysosomal-mediated degradation via a specifically controlled trafficking pathway through specialized organelles derived from the fusion between endosomes and autophagosomes (namely “amphisomes”). Furthermore, we have demonstrated that these organelles are also mediators of the DC-mediated innate and adaptive immune responses, which led us to name them “immunoamphisomes”.

Importantly, enhanced cell-to-cell HIV-1 transmission was observed in autophagy-deficient DC suggesting that the early events of viral entry critically influence the subsequent steps of viral polarization and transfer.

We will determine the mechanisms underlying specific receptor-mediated autophagolysosomal targeting, identify critical receptor-associated cellular factors and try to elucidate the connection between these organelles and intracellular immune compartments (TLR-containing vesicles, MHC-II compartments…). Our studies will provide valuable information on the mechanisms regulating virus/receptor internalization as well as the identification of key players in viral endocytosis and cellular trafficking and their impact on the late steps of viral transfer. Identification of potential “druggable” targets are of importance to tackle early events of HIV-1 and therefore impeding cell-to-cell viral transmission.

Laure Papin (Ph.D student), Fabien Blanchet (CR1), Lucile Espert (CR1). CPBS
Summary Cryptococcal meningitis (CM) is a severe opportunistic infection in human immunodeficiency virus (HIV) infected patients. In the Ivory Coast, despite the availability of antiretroviral treatment (ARV), this infection is still prevalent. The study investigates the genetic diversity of 363 clinical isolates of Cryptococcus from 61 Ivorian HIV positive patients, the occurrence of mixed infections and the in vitro antifungal susceptibility of the strains. Serotyping was performed via LAC1 and CAP 64 gene amplification. Genotyping was performed using the phage M13 core, (GACA)4 and (GTG)5 primers and restriction fragment length polymorphism (RFLP) analysis of the URA5 gene. We demonstrated the presence of the following 3 serotypes among the 363 isolates in the population studied: A (n=318; 87.6%), AD (n=40; 11%) and B (n=4; 1%). Using PCR fingerprinting with the microsatellite-specific primer M13 and minisatellite-specific primers (GACA)4 and (GTG)5, we grouped the isolates into 56 molecular subtypes. We discovered a high frequency (39.3%) of mixed infections, with up to two different genotypes per sample. None of the isolates were resistant to amphotericin B. Only 0.3% and 0.8% of the isolates were resistant to fluconazole and flucytosine, respectively. No correlations were found among the genotype and resistant strains. This study revealed the high genetic diversity of Cryptococcus strains from the Ivory Coast, the occurrence of mixed infections and a high antifungal susceptibility for the majority of Ivorian cryptococcal isolates. Key words: Cryptococcosis, genetic diversity, genotyping, mixed infection, antifungal susceptibility, Ivory Coast.

Fulgence K. Kassi1, Pascal Drakulovski2, Virginie Beller2, Donika Krasteva2, François Gatchitch2, Adama Doumbia4, Gisèle A. Kouakou4, Eric Delaporte3, Jacques Reynes3, Michèle Mallié2, Hervé J.E Menan1 and Sebastien Bertout2. 1Université Félix Houphouët Boigny, UFR Pharmacie, Laboratoire de Parasitologie et de Mycologie – CeDReS (Centre de Diagnostic et de Recherche sur le SIDA), CHU de Treichville, Abidjan. 2UMI 233 IRD-UM INSERM U1175 Laboratoire de Parasitologie et de Mycologie, UFR Pharmacie, 15 Av. C. Flahault, BP 14491 34093 Montpellier Cedex 5, France. 3UMI 233 Service des Maladies Infectieuses et Tropicales, CHU Gui de Chauliac, Montpellier, France. 4Service des Maladies Infectieuses et Tropicales, CHU de Treichville, Abidjan, Côte d’Ivoire.
N°35 – Gabriel ESPINOSA CARRASCO
Dynamics of a T cell mediated autoimmune attack to the pancreas in real time

Background and aims: Type 1 diabetes results as a failure of the mechanisms that maintain immune tolerance. Both, CD8+ and CD4+ T cells override tolerance and cooperate to progressively destroy the Beta-cells of the pancreas. Interestingly, it has been recently shown the in vivo dynamics of immune cell activation in the lymph nodes. However, the dynamics of Beta-cell destruction and islet infiltration cellular interactions involved in vivo have not been characterized and are essential to understand the mechanism involved in Beta-cell killing by immune cells. With a T1D transgenic mouse model and intra-vital 2-photon microscopy, we have investigated an autoimmune attack mediated of both CD8+ and CD4+ T cells, by directly visualizing the interactions between individual CD4+/CD8+ T cells and Beta-cells. Our results showed, for the first time, the in vivo CD8+ and CD4+ T cells cooperation to destroy pancreatic Beta-cells in a T1D transgenic mice model.

Conclusions:
1) Islet-specific CD8+ and CD4+ T cells become activated in the pancreatic lymph nodes. Effector T cells, them, infiltrate the pancreas inducing Beta-cell destruction and diabetes.
2) Unlike CD8+ T cells, CD4+ T cells, in the absence of CD8+ T cells, infiltrate the pancreas inducing peri-insulitis but not diabetes.
3) Intra-islet CD8+ T cells displayed significant reduced mean velocity and higher arrest compared to CD8+ T cells in exocrine tissue, as expected, and consistent with interactions leading to Beta-cell killing.
4) Intra-islet CD4+ T cells also displayed significant reduced mean velocity and higher arrest compared to CD4+ T cells in exocrine tissue, probably due to prolonged interactions with Beta-cells and antigen-presenting cells accumulated in infiltrated pancreatic islets.
5) CD4+ T cells display significant higher arrest compared to CD8+ T cells in the exocrine tissue, due to prolonged interactions with antigen-presenting cells. In vivo injection of MHC-II blocking antibody reverse this situation.

Gabriel Espinosa-Carrasco¹,², Pierre Fontanaud², Patrice Mollard², Marie Schaeffer² and Javier Hernandez¹. ¹Inserm U1183, Institute for Regenerative Medicine and Biotherapy. ²Inserm U1191, Institut de génomique fonctionnelle. Montpellier, France.
Flash Talks and Poster Session 2
Integrated approach of gamma-irradiation on Caenorhabditis elegans : from DNA to proteins

Chronic-exposure to ionizing-radiation is a major environmental issue. However, most of studies are relative to acute-exposure. For a same cumulated-dose, the observed effects on organisms are distinct after acute and chronic-radiation, meaning possible different underlying mechanisms. After huge acute dose of gamma rays, at equal DNA damage, it has been demonstrated that differential radiosensitivity between species could be explained by a differential proteome protection system particularly against oxidation and protein carbonylation (PC). It’s therefore necessary to acquire specific data about effects and molecular mechanisms induced by chronic-irradiation, particularly on protein damage. For this approach C.elegans appears to be a powerful tool as its life cycle is short for chronic exposure and it is fully sequenced for mechanism elucidation.

The objectives of the project are 1/ to assess the sensitivity of PC (rate and nature) after acute and chronic-radiation-exposure, 2/ to discriminate these two irradiation modes in an integrated context, linking PC to other molecular effects (DNA damage and repair, proteolytic activity) and to individual damage (reproduction).

PC-rate on different stage of worms and after acute-irradiation (2.5 and 75Gy) was assessed by methodologies recently developed whereas differential carbonylome (after 2.5Gy acute-irradiation) was performed via collaboration (Medils).

The first results showed that PC level depends on the nematode life cycle with a minimum of PC level for young adult stage (sexual maturity). Kinetics of PC production performed at this stage after 2,5 and 75 Gy acute-exposures showed a maximum PC rate 3h post irradiation. Finally, the study of carbonylomes by 2D analysis showed 126 different carbonylated protein spots between controls and exposed, 15 has been identified by mass spectrometry.

The same methodology will be performed after multidose acute- and chronic-irradiation, to characterize PC sensitivity between 0 and 200Gy and finally compare the two-irradiation-modes through PC and other molecular and individual parameters.

Cécile Dubois, Sandrine Frelon, Catherine Lecomte, Simon Galas, Sébastien Pyr dit Ruys, Mira Kuzmic, Romain Ladouce, Luc Camoin.
Cardiac automaticity is generated by specialized “pacemaker” myocytes located in the sino-atrial node: a thin tissue situated in the right atrium. Pacemaker cells express a specific set of ion channels that underlie heart automaticity. Among them, Cav1.3 L-type and Cav3.1 T-type calcium channels and f-(HCN) channels are thought to be important actors of heart pacemaking. In spite of their importance, their potential functional interaction in promoting pacemaking is unknown. To clarify this point we studied the role of Cav1.3 and Cav3.1 channels using telemetric recording of electrocardiograms (ECGs) in freely moving animals from three genetically modified mouse models: Cav3.1−/−, Cav1.3−/− and Cav1.3−/−/Cav3.1−/−. We analyzed ECG by measuring the heart rate (HR) and quantify the frequency of II-degree atrioventricular blocks (AVBs) under different pharmacological conditions. Cav1.3−/−, Cav3.1−/− and Cav1.3−/−/Cav3.1−/− mice showed a reduction in basal HR of 40 %, 10 % and 33 % respectively, compared to control animals. Similar reduction percentage in Cav1.3−/− and Cav1.3−/−/Cav3.1−/− suggests functional interactions between the two channels. We did not observe AVBs episodes in ECG recordings control and Cav3.1−/− mice. On the contrary, Cav1.3−/− and Cav1.3−/−/Cav3.1−/− showed high frequency of AVBs underlying important atrioventricular conduction dysfunctions. To study the impact of the autonomic nervous system (ANS) activity in knock-out animals, we co-injected atropine (0,5mg/kg) and propranolol (5mg/kg) to pharmacologically inhibit the ANS input. The injection strongly decreased HR and AVBs in mutant mice. These observations suggest that atrioventricular dysfunction recorded in control conditions in the genetically modified mouse models was strongly dependent on the activity of ANS. Injection of Ivabradine (6 mg/kg), a well-known f-(HCN) blocker, reduced HR of about 50% in all mice tested. Strikingly, Ivabradine injection in Cav1.3−/−/Cav3.1−/− did not stop the HR, suggesting that other mechanisms out of L-type and T-type Ca channels and HCN4 channels are involved in cardiac automaticity.  

M. Baudot, P. Mesirca, I. Bidaud, A. Chung You Chong, S. Barrère and M.E. Mangoni. Département de Physiologie, Institut de Génomique Fonctionnelle, LabEx ICST, UMR-5203 CNRS, INSERM U661, Université de Montpellier.
Interaction of Fe3O4@MSN nanoparticles with cells membranes

Mesoporous silica nanoparticles (MSN) are promising materials as drug delivery systems or nanovectors. These biocompatible materials associated to magnetic properties form interesting multifunctional Fe3O4@MSN platforms. In this work we investigate interactions of Fe3O4@MSN with the cellular membrane as a function of nanoparticles (NPs) surface coverage.

The synthesis of the nano-objects has been optimized to obtain Fe3O4 as a magnetic core, surrounded by a mesoporous silica shell with a size of 100 nm. The silica pores, with a diameter of 3 nm, allow a large surface area, with silanol groups at the surface. Pristine Fe3O4@MSN have been coated with a zwitterionic lipid bilayer or grafted with polyethylene glycol (PEG). Pristine and coated Fe3O4@MSN have been dispersed and characterized in different media. Fe3O4@MSN dispersion was largely dependent on medium composition (ionic strength and protein content) and MSN coating (lipid bilayer or PEG).

MTT cell viability assay was performed on HepG2 cell culture model. Results show a slight decrease in cell viability at 50 g mL⁻¹ for pristine and DMPC NPs. Pristine NPs are the more cytotoxic, followed by DMPC NPs and then PEG NPs. The cell uptake of pristine, lipid coated and PEG grafted Fe3O4@MSN have been investigated by TEM observation after 6h of exposure. The NPs with three different surfaces penetrate the cells. A supported lipid bilayer (SLB) is used as biomimetic membrane model to investigate Fe3O4@MSN - cell membrane interactions. Using surface plasmon resonance, real-time interactions were monitored. Furthermore, electrical properties of the membrane were characterized in the presence of NPs.

This study associating physicochemical properties, cell effects and interaction with membrane models allows a better understanding of the factors influencing MSN cell membrane interactions. Thanks to the SLB model: dispersion media, Fe3O4@MSN coating, and transmembrane potential were found to influence MSN cell membrane interactions.

E. Rascol¹, C. Pisani¹², J. Nyalosaso¹, C. Dorandeau¹, X. Dumail¹, C. Charnay¹, Y. Guari¹, M. Gary-Bobo³, M. Maynadier⁵, J. Lai Kee Him⁴, P. Bron⁴, M. Garcia³, O. Prat², J. Armengaud², JM. Devoisselle¹, J.Chopineau¹. ¹UMR5253 CNRS/UM/ENSCM, Institut Charles Gerhardt de Montpellier. ²SBTN, CEA Marcoule, Bagnols-Sur-Cèze. ³UMR5247 CNRS/UM, Institut des biomolécules Max Mousseron, Montpellier. ⁴Centre de biochimie structurale CNRS UMR 5048 / UM / INSERM U 1054, Montpellier. ⁵Nanomedsyn, Montpellier, France.
N°39- Quentin SEISEL

Screening and vectorization of therapeutic peptides for cystic fibrosis treatment

Cystic fibrosis (CF) is the most common fatal genetic disorder in populations of European descent. CF is due to loss-of-function mutations in CFTR (cystic fibrosis transmembrane conductance regulator), an epithelial ion channel strongly involved in fluid and ion homeostasis. Its activity being required for airway mucociliary clearance, CF patients suffer from airway obstruction and chronic infection.

One of the three known functional defects associated with the most prevalent mutation, DF508-CFTR, concerns the stability of the protein at the apical membrane of endothelial cells. We decided to target the PDZ domain of the CAL (CFTR-associated ligand) protein, which is a key regulator of CFTR trafficking. In 2010, our group has reported the development of a CAL inhibiting peptide (iCAL), showing an 11% increase of CFTR activity in cellulo. However, optimization of the peptide sequence in terms of metabolic stability and cellular delivery are still necessary to obtain more efficient inhibitors. iCAL sequence modulations will be performed using SPOT synthesis, which enables peptide-protein interactions (PPI) screening via cellulose-supported parallel synthesis of thousands of peptides. In the meantime, increase in iCAL cellular internalization will be carried out by conjugating the inhibitor to a cell-penetrating peptide (CPP).

Here, we will present the optimisation of the “method of inverted peptide”, a variant of the SPOT synthesis required to screen the PDZ domain of the CAL protein. This process is thoroughly studied to obtain the best signal-to-noise ratio for the PPI detection. In parallel, different CPPs have been conjugated to the iCAL sequence and their interaction with large unilamellar vesicles (LUV) representing a model of plasma membrane are studied.

Altogether, the presented results will push forward the development of more selective and specific iCAL inhibitors as new therapeutics to treat the symptoms of CF pathology.

Quentin Seisel*, Prisca Boisguerin. Centre de Recherche de Biochimie Macromoléculaire, CNRS-UMR 5237, 1919 route de Mende, 34293 Montpellier, France.

*Correspondence: quentin.seisel@crbm.cnrs.fr
N°40 – Loubna DRISSENNEK
S100A10 silencing promoted apoptosis of epithelial cells resulting probably in implantation failure process

Many biomarkers of human endometrial receptivity have been previously reported. However, few studies i) investigated the relevance of their biomarkers in patients with multiple implantation failures, and ii) performed functional analyses to identify their role(s) and function(s) during the implantation window of fertile patients. In this study, we investigated function(s) of one endometrial receptivity biomarker, a S100A10 family member expressed in epithelial cells, using shRNAs. We analyzed the S100A10 protein extinction impact in relation to apoptosis. We also assessed mRNA expression level of S100A10 during the implantation window of 27 patients with multiple implantation failures after in vitro fertilization. In epithelial cells, serum withdrawal induced apoptosis only in shRNA S100A10 epithelial cells compared with scrambled shRNA cells. 50% of cells were positive for activated caspase 3 and fragmented nuclei according to the DAPI staining in shRNA S100A10 epithelial cells compared with scrambled shRNA cells. In addition, patients with multiple implantation failures were characterized by a down-regulation of S100A10 mRNA expression level compared with fertile patients (p-value=0.0012). As apoptosis plays a central role in the endometrium during the period of establishment of the implantation window, a deregulation of this signaling pathway due to the down-expression of the S100A10 mRNA can explain implantation failures in multiple implantation failures.

Drissennek L, Antoine Y, Bissonnette L, Haouzi D, Hamamah S. ¹CHU Montpellier, Institut de Médecine Régénératrice et de Biothérapie, Hôpital Saint-Eloi, Montpellier, F-34295 France. ²CHU Montpellier, Département de Biologie de la Reproduction, Hôpital Arnaud de Villeneuve, Montpellier, F-34295 France ; ³INSERM, U1203, Montpellier, F-34295 France. ⁴Université Montpellier1, UFR de Médecine, Laboratoire ‘Développement embryonnaire précoce humain et pluripotence’, Montpellier, F-34000 France; OVO Fertility, 8000 Boulevard Decarie #100, Montréal, Canada H4P 2S4.
N°41 – Mathilde LACÔTE

Sarcoplasmic Reticulum Ca2+ leak and mitochondrial reactive oxygen species: the auto-amplification loop

Part of the Ca2+ released by the Sarcoplasmic Reticulum (SR) through the type-2 ryanodine receptor (RyR2) is taking up by the mitochondria to favor ATP production. Although, mitochondrial Reactive Oxygen Species (ROS) production may affect RyR2 open probability, the consequences of a primary RyR2 dysfunction on mitochondrial metabolism remain unknown. Thus, the aim of the present study was to determine if an increase in SR Ca2+ leak alters mitochondrial function. To answer this question, we used a model of calstabin2 (FKBP12.6) deficient mice, a small protein that stabilizes RyR2 close state. Under confocal microscope we observed that calstabin2 deficiency increases diastolic SR Ca2+ leak (Ca2+ sparks) as well as Ca2+ transients amplitude without any change in SR Ca2+ load. This altered Ca2+ homeostasis is associated with a progressive increase in RyR2 oxidation and open probability. On the other hand, the maximal respiration capacity and the mitochondrial content were increased. However the dynamic mitochondrial calcium movement, recording under whole cell patch-clamp technique, was reduced and mitochondrial ROS production measured with MitoSox red was enhanced. In summary, our data demonstrate the existence of an auto-amplification loop between SR Ca2+ leak and mitochondrial ROS production: altered RyR2 function disturb mitochondrial ability to take up Ca2+, increasing mitochondrial ROS production on a beat-to-beat basis and finally favoring RyR2 oxidation.

Lacôte Mathilde¹, Saint Nathalie¹, Thireau Jérôme¹, Roy Jérôme¹, Angebault-Prouteau Claire², Scheuermann Valérie¹, Farah Charlotte¹, Cazorla Olivier¹, Lacampagne Alain¹, Fauconnier Jérémy¹. ¹INSERM U1046, UMR CNRS 9214, Université de Montpellier. ²INSERM U1051, Institut des Neurosciences, Montpellier.
Regions 1.2 and 3.2 of the RNA Polymerase σ Subunit Promote DNA Melting and Attenuate Action of the Antibiotic Lipiarmycin

Initiation of RNA synthesis by bacterial RNA polymerase (RNAP) requires melting of promoter DNA, which is nucleated by the σ subunit during formation of the “open” promoter complex (RPo). The antibiotic lipiarmycin (Lpm) inhibits promoter melting by blocking access of the template DNA strand to the RNAP active-site cleft.

Here we show that Escherichia coli RNAP holoenzymes containing either housekeeping σ70, with a deletion in the region 3.2, or the stationary phase σS subunits exhibited hypersensitivity to Lpm and increased cold sensitivity of RPo formation. Similar effects were produced by mutation located ~60 Å away from the Lpm binding site within σ70 region 1.2, controlling −10 promoter element recognition. Our data suggested that template strand single-stranded DNA competes with Lpm for binding to RNAP and that σ70 regions 1.2 and 3.2 attenuate Lpm action by promoting DNA duplex opening.

Zakia Morichaud, Laurent Chaloin and Konstantin Brodolin
N°43 – Alexandra POMMIER

Role of miRNA in Cystic Fibrosis pathology

Objectives: Alteration of miRNA profiling in CF is now well established. miRNA expression has been assessed in various CF models, mainly by quantification of preselected miRNAs including the profiling platform TLDA (TaqMan Low Density Array). Because sensitivity and specificity differ depending on the miRNA profiling platform, we decided to investigate miRNA expression profiles by using both TLDA and unbiased sequencing approaches on three ALI epithelium models, taken from non-CF and CF airways.

Material and methods: MicroRNA profiling platforms including both TLDA and microRNA sequencing (MiSeq, Illumina) approaches were employed to determine an inventory of miRNAs expression. Total RNAs were extracted from nasal cells, polyps and bronchial cells, cultured in Air-Liquid Interface (ALI, n=4 per condition). To test miRNAs effect, luciferase reporter assays were conducted. Level of expression of CFTR gene and miRNAs was assessed by RT-qPCR.

Results: In this study, we reported the deregulation of 41 miRNAs found in at least two models as miR-27b, miR-100*, miR-27a-5p, and miR-449/miR-34, two microRNA families previously described as key regulators in ciliogenesis. Among the deregulated miRNAs, we first selected those predicted to target CFTR-3’utr including miR-449, miR-27a-5p and miR-100*. By using gene reporter assays, impact of these miRNAs on CFTR-3’utr was confirmed. In addition, six miRNAs target TTP (Tristetraprolin), an AU-rich binding protein described to directly impact IL-8 mRNA stability in CF lung epithelial cells. As we had previously demonstrated the presence of AU-rich elements in CFTR-3’utr, we next decided to determine the role of TTP on CFTR gene expression. Introduction of siRNA directed against TTP negatively influenced the CFTR-3’utr stability by using luciferase gene reporter assays and downregulated endogenous CFTR mRNA level.

Conclusion: These data led to the identification of new regulatory players in CF physiopathology and new regulators in the regulation of CFTR gene expression.

A. Pommier¹, J. Varilh², J. Bonini¹, R. Chiron³, E. Brochiero⁴, M. Koenig¹-², M. Claustres¹-², M. Taulan-Cadars¹. ¹Laboratory of Genetics of Rare Diseases EA7402, University of Montpellier, France. ²Laboratory of Molecular Genetics, CHRU Montpellier, France. ³Department of Respiratory Diseases, CHRU Montpellier, France. ⁴Department of Molecular and Integrative Physiology, University of Montréal, Canada.

N°44 – Fanny PINEAU
Identification of epigenetic predictive biomarkers of lung disease severity in cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive genetic disease mainly characterized by airway obstruction, mucus accumulation, respiratory infection and inflammation. It is the most common life-threatening recessive genetic disease in the Caucasian population. Morbidity and mortality are mainly due to lung disease, which is variable among CF patients, even for those having the same genotype. Contributing factors are mutations in CFTR (the disease-causing gene), modifier genes, but also environmental factors and epigenetics. We hypothesized that DNA methylation contributes to lung disease variability. In a Genome Wide Analysis (GWA) with 450K BeadChip Array (Illumina), we profiled DNA methylation in DNA extracted from nasal epithelial cells (NEC) of 32 CF patients (pulmonary severity: mild, intermediary or severe) and 16 healthy controls. We identified 638 differentially methylated genes (DMG) in CF patients compared to controls, and 116 DMG in severe compared to mild CF patients. Gene ontology analyses of these DMG highlighted cellular processes relevant to CF, i.e cell adhesion and cell motion. Interestingly, 87 out of 638 DMG were differentially expressed in publicly available NEC transcriptomic data. Currently, we are validating and replicating these methylation data to find new genes that may be responsible for lung disease variability in CF. Six DMG have been validated and/or replicated, using DNA bisulfite conversion and pyrosequencing with PyroMark Q24 (Qiagen). Our main goal is to identify epigenetic predictive biomarkers of lung disease severity among the DMG in mild versus severe CF patients. A second objective is to characterize a non-invasive model suitable for the analysis of these biomarkers. Sputum is easy to collect in a non-invasive way, and is already used to analyze airway inflammation in CF patients. It might be a good model for biomarkers analyses as part of the follow-up of CF patients.

F. Pineau, M. Magalhães, M. Thomasset, R. Chiron, J. Tost, I. Rivals, L. Mely, S. Leroy, M. Murris, D. Caimmi, I. Vachier, M. Claustres, A. De Sario. 1EA Université de Montpellier. 2Montpellier Hospital. 3CNG - CEA, Evry. 4ESPCI Paris. 5Hyères Hospital. 6Nice Hospital. 7Toulouse Hospital.
DNA synthesis is initiated at defined sites along the genome called DNA replication origins. Potential origins are established in G1-phase of the cell cycle, a process known as replication licensing. Biochemically, origins are recognized by the ORC complex, a conserved multisubunit ATPase. Along with the essential co-factors Cdc6 and Cdt1, the ORC complex catalyzes the chromatin loading of the replicative helicase, the MCM2/7 complex, in an inactive state. During S-phase, CDK and DDK kinases activate a subset of origins, resulting in DNA unwinding and initiation of DNA replication. Our group studied the interactome of origin factors (ORC, LRWD1, Cdc6, Cdt1) in an unbiased manner through a proteomic approach and identified an uncharacterized RING-type E3 ubiquitin ligase that we called Obi1 (for ORC ubiquitin-ligase 1). Earlier experiments performed in human cells show that Obi1 is an important regulator of origin activation. To get further insights into the function of Obi1, we decided to utilize the Xenopus egg extract system. This model is the only in vitro system able to recapitulate all the steps involved in DNA replication. First, we have generated several polyclonal antiserum raised in rabbits against two different antigens derived from Xenopus Obi1 homolog. These antibodies were purified against the immunogen. Using these unique tools, we could confirm that Obi1 is expressed in Xenopus egg extracts. Obi1 was found to associate with chromatin and form high molecular complexes. We are now setting up the experimental conditions for immunodepletion to perform loss-of-function experiments. In these experiments, Obi1 is depleted from Xenopus egg extracts using our purified antibodies and the replication capacity of the extract is evaluated. In addition, we will study the ubiquitylation of chromatin proteins during replication. This project will provide crucial information on the regulation and function of Obi1 in DNA replication.

Joelle NASSAR, Antoine AZE, Stéphane BOCQUET, Magali KITZMANN, Marcel MÉCHALI, and Philippe COULOMBE
N°46 – Moïra ROSSITTO

Risks of in utero Non Steroidal Anti-Inflammatory Drugs and acetaminophen exposure on the early testis development

The non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen (ACE, paracetamol) are used worldwide to reduce mild to moderate pain, fever... Some medications are available over the counter without a prescription and are often used in self-medication. In Europe and the United States, more than 50% of pregnant women take analgesic drugs. These medications are not recommended only after 5 months of pregnancy. Very few studies have focused on the putative effects of these drugs on the formation of embryonic gonads, knowing that the gonads develop in humans in the first trimester of pregnancy (between 6 and 8 weeks). The targets of NSAIDs and ACE are the cyclooxygenases (COX), the key enzymes in the synthesis of prostaglandins from arachidonic acid. Prostaglandin D2 (PGD2) is involved in different stages of development and maturation of embryonic testis at the somatic and germinal levels.

The aim of this study is to evaluate the impact of in utero exposure to ACE and NSAIDs on the mouse testis development.

Moïra Rossitto, Pascal Philibert, Candice Marchive, Naouëlle Bouabas, Francis Poulat & Brigitte Boizet-Bonhoure.
A good way to increase the efficiency of rescuing virus

Reverse genetic has been widely used to rescue virus. For some virus, the system of reverse genetic consists of different kinds of plasmids. For example, the system for paramyxovirus is made up by four plasmids including viral full genome, N, P and L gene, respectively. It is necessary to transflect all of them into the same cell to rescue virus. In addition, some paramyxovirus, such as lentogen Newcastle disease virus (NDV), could not amplify without stimulation by exogenous trypsin, which makes the system is lower efficient. Therefore, more cells are transfected by all kinds of plasmids to get more viral particles and then the chance for successfully getting virus is higher. In here, the kinds of plasmid of NDV’s rescuing system have been decreased from four to two. This novo method was not just let the rescuing system simpler, but also increased the rescuing efficiency based on mini-genome and full-genome test.

Haijin Liu¹, Renata Servan de Almeida¹, Patricia Gi¹, Emmanuel Albina¹. ¹CIRAD, UMR CMAEE, F-34398 Montpellier, France.
Objecitives- Recently, we identified a colonizing Staphylococcus aureus strain with a low virulence isolated from diabetic foot ulcers. The main characteristic of this strain is the presence of a phage (Rosa-like) inserted near the locus isd, the main heme iron-uptake system. The aim of this study was to evaluate the stability of this phage and its impact in the metabolism of this strain.

Methods- We develop an in vitro model miming the conditions encountered in DFU consisting to cultivate during 24 weeks S. aureus strains by successive inoculations in environments with: sugar 2%-10%, antibiotics at 0.25-0.75x MICs (linezolid, vancomycin, cloxacinil), anaerobia, and minimal medium. The presence/absence of the phage was tested by PCR. Transcriptomic analysis (microarray, qRT-PCR) was performed to study the global behavior of this strain in presence/absence of iron. Quantification of siderophores production, evaluation of biofilm formation and adhesion assays on HaCaT cells were also performed.

Results- Our results showed that the ROSA-like phage is stable and cannot be excised whatever the stress condition tested after 24 weeks. Transcriptomic analysis revealed that the phage derepressed the activity of the transcriptional regulator Fur, reduces significantly the growth of the strain, biofilm formation and blocks iron uptake. In a low-iron environment, global metabolism of the colonizing strain showed that 37% and 29% of genes were over- and under-expressed, respectively. The survival of bacteria was due to the activation of accessory pathways (fructose, lipid and nickel). The colonizing behavior was explained by a downregulation of the adhesines and virulence genes. The decrease of adhesion was confirmed on HaCaT cells.

Discussion- With this study we describe for the first time a mobile element present in S. aureus, which modulates the relationship of the bacteria with its human host by attenuating its virulence. Such discovery is an important advance in commensalism understanding. Keywords: Staphylococcus aureus, Rosa-like phage, metabolism, iron.
Adoptive anti-tumor T cell immunotherapies have demonstrated promising results, notably for the treatment of chemotherapy-resistant cancers. Specifically, CD8+ T lymphocytes, genetically modified to express a chimeric antigen receptor (CAR) against the CD19 antigen, have been used to successfully treat refractory B leukemias/lymphomas. However, recent studies also highlight the potential role of other immune subsets in improving anti-tumor immunity. In order to fully explore the potential of different T cell subsets as well as other leukocytes armed with an anti-tumor CAR, we generated a preclinical transgenic mouse model expressing a CAR specific for the Her2 (ERB) tumor antigen in all immune subsets. Using this model, wherein the Her-CAR is expressed under the control of the vav pan-hematopoietic promoter, we found that Her2-CAR T cells exhibit Her2-specific immune responses, monitored as a function of cytokine secretion as well as cytotoxicity. Furthermore, we are assessing whether the metabolic features of Her2-CAR immune cells regulate their relative persistence, proliferation, differentiation and anti-tumor potential. In particular, we aim to assess the cellular metabolism of Her2-CAR immune cells under alternative nutrient availability and to segregate Her2-CAR cells on the basis of their glycolytic state, identified by surface Glut1 glucose transporter levels. The capacity to modulate the function of anti-tumor immune cells through a metabolism-based approach opens new avenues for optimizing adoptive immunotherapies.

Carmen SM Yong1,2, Christel Devaud3,5, Phillip K Darcy1,4,5, Michael H Kershaw1,4,5, Valerie Dardalhon2,5, Naomi Taylor2,5. 1Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Victoria 3010, Australia. 2Institut de Génétique Moléculaire de Montpellier, CNRS, UMR 5535, Université de Montpellier, F-34293 Montpellier, France. 3Institut de Recherche en Santé Digestive, Université de Toulouse, INPT, INRA, INSERM UMR1220, UPS, France. 4Department of Immunology, Monash University, Prahran Victoria 3181 Australia. 5These authors contributed equally to this work.
Cell surface Glut1 levels distinguish human T lymphocyte subsets with distinct effector functions

T lymphocyte activation requires the generation of sufficient energy to support new biosynthetic demands. Following T cell receptor (TCR) engagement, these requirements are met by an increased glycolysis, due, at least in part, to induction of the Glut1 glucose transporter. As Glut1 is upregulated on tumor cells in response to hypoxia, we assessed whether surface Glut1 levels regulate the antigen responsiveness of human T lymphocytes in both hypoxic and atmospheric oxygen conditions. Notably, Glut1 upregulation in response to TCR stimulation was significantly higher in T lymphocytes activated under hypoxic as compared to atmospheric oxygen conditions. Furthermore, TCR-stimulated human T lymphocytes sorted on the basis of Glut1-Lo and Glut1-Hi profiles maintained distinct characteristics, irrespective of the oxygen tension. Glut1-Hi lymphocytes exhibited augmented proliferation, an augmented CD8/CD4 ratio and increased effector function. Thus, our data showing that Glut1 acts as a gauge of T lymphocyte function fosters the development of novel therapeutic strategies for manipulating T cell responses in immune-related pathologies such as autoimmunity, infection and cancer.

Maria Matias*, Gaspard Cretenet*, Isabelle Clerc, Severine Loisel, Marco Craveiro, Leal Oburoglu, Sandrina Kinet, Cédric Mongellaz, Valérie Dardalhon* and Naomi Taylor*. *equal contribution Institut de Génétique Moléculaire de Montpellier, Centre National de la Recherche Scientifique UMR5535, Université de Montpellier, F-34293 Montpellier, France
Coxiella burnetii, the etiological agent of the zoonosis Q fever, replicates inside host cells within a large vacuole with autolysosomal characteristics. The development of this compartment is mediated by the translocation of bacterial effectors by a Dot/Icm, type 4 secretion system, which interfere with a number of host membrane trafficking pathways. Our team has generated and screened the first library of Coxiella transposon mutants, leading the identification of a significant number of candidate virulence determinants and effector proteins. One of these, CvpF, is a newly identified Coxiella translocated effector that localises at the Coxiella-containing vacuole (CCV). Transposon insertions in cvpF have consequences on the size of CCVs, which is significantly reduced as compared to that of CCVs generated by wt Coxiella. Ectopically expressed CvpF localises at CCVs as well as intracellular compartments positive for LAMP1 and PI(3)P. Membrane targeting of the bacterial effector is mediated by a domain encompassed between amino acids 370 and 500, which shares homologies with the mammalian Vps16, involved in the biogenesis of multivesicular bodies (MVBs). Additionally, yeast 2-hybrid screening revealed a potential interaction with the host small GTP-ase Rab26, which is seemingly confirmed by immunofluorescence studies. Interestingly, CvpF expression recruits Rab26 at host cell membranes. How CvpF controls the MVBs trafficking machinery and Rab26 for CCVs biogenesis is currently being investigated.

Fernande A Siadous¹, Eric Martinez¹, Matteo Bonazzi¹. ¹CPBS
Role of nitrosative stress in the anti-mycobacterial properties of the KdpF membrane peptide

Membrane peptides appear as an emerging class of regulatory molecules, which can interact with membrane proteins and interfere with their activity. Recent studies have identified membrane peptides that modulate bacterial virulence. The 30 amino-acid long KdpF peptide is supposed to be a subunit of the KdpABC potassium transporter. In a previous study, we have shown that overexpression of the KdpF membrane peptide in Mycobacterium bovis BCG resulted in reduced intramacrophage growth and altered cording morphology. To decipher the relation between KdpF overexpression and intramacrophage growth, we have tested the sensitivity of overexpressing KdpF mycobacteria to several stresses. These bacteria display a significantly higher sensitivity to oxidative stresses (Nitrosative stress, SNAP). Importantly, the treatment of macrophages with inhibitors of nitrogen intermediates (RNI) partially suppressed the intramacrophage growth defect of KdpF overexpressing bacteria. Moreover, the production of nitric oxide (NO) by macrophages infected with KdpF overexpressing mycobacteria is increased comparatively to a control strain. Preliminary data indicate that addition of a synthetic KdpF peptide to wild-type M. bovis BCG also increases the sensitivity to oxidative stress (SNAP), thus mimicking the effect of endogenous KdpF overexpression. Taken together, our results suggest that M. bovis BCG strain overexpressing KdpF is more sensitive to nitrosative stress, which in turn reduces the intra macrophage survival.

Mariana Rosas Olvera¹, Eric Vivès¹, Virginie Molle¹, Anne-Béatrice Blanc-Potard¹, Laila Gannoun-Zaki¹.
¹UMR-CNRS 5235, DIMNP, Université de Montpellier, case 107, Place Eugène Bataillon, 34095 Montpellier cedex 5.
Brucella is the causative agent of brucellosis, the major bacterial zoonosis worldwide. In the last years, new species and atypical strains, as B. microti, B. inopinata and isolates from bullfrog, were described. These strains are more acid-resistant than the most well-known pathogenic Brucella species (B. abortus, B. melitensis, B. suis). In Escherichia coli, the glutamate decarboxylase (GAD)-dependent system and the AR2-Q system, based on deamination of glutamine, are most efficient for extreme acid resistance (AR). First, our team has demonstrated that the GAD system of B. microti allows survival at pH 2.5 and contributes to murine infection by oral route; second, this system is functional only in atypical Brucella strains of ancestral origin, but not in the classical species, which are mostly adapted to livestock and human hosts. The aim of my PhD project is to study the role of the glutaminase system in AR of Brucella spp. Indeed, our recent results indicate that the new strains possess an intact glutaminase-encoding gene (glsA) and are AR in the presence of glutamine. In Brucella, the gadB and gadC genes encoding the GAD system are located just upstream of and in the same orientation as glsA and hdeA, encoding a chaperone involved in the survival of E. coli and of B. abortus in acidified media. By RT-PCR analysis, we have demonstrated that all these genes form an operon. The role played by each of them in extreme AR will be studied by constructing and analyzing the phenotypes of the corresponding mutant strains of B. microti. Our results suggest that the GAD and glutaminase systems may contribute to improving the adaptability of new species of Brucella to certain natural habitats and/or to the gastrointestinal tract of their hosts.

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Freddi L1, Damiano MA1, Al Dahouk S3, Köhler S1, De Biase D2, Occhialini A1. 1CPBS, Centre d’études d’agents Pathogènes et Biotechnologies pour la Santé, CNRS – University of Montpellier, Montpellier, France. 2Istituto Pasteur-Fondazione Cenci Bolognetti, Dipartimento di Scienze e Biotecnologie Medico-Chirurgiche, Sapienza University of Roma, Latina, Italy. 3Federal Institute for Risk Assessment, Berlin, Germany.
EBI2 interacts with the HIV co-receptor CCR5 and enhances HIV infection

CCR5 is a G protein-coupled receptor (GPCR) used by the Human Immunodeficiency Virus (HIV) to infect CD4+ lymphocytes. As heterologous interactions among GPCR are well-documented, we questioned whether we could find GPCRs that would interfere with the HIV-1 cycle when co-expressed with CCR5 at the surface or CD4+ cells.

We first identified all the GPCR co-expressed with CCR5 in circulating CD4 T cells by multi quantitative RT-PCR. We screened the 40 most expressed GPCRs by individual transfection in CD4+ CCR5+ HEK293 cells followed by a HIV replication test in these cells. This approach let us identify several GPCR candidates. These candidates were first assessed for their ability to interact with CCR5 at the membrane of HEK293 cells, using a TR-FRET technology. We then questioned for the ability of the CCR5 interacting GPCRs to modify HIV-1 cycle. For this, we established two HOS cell lines expressing or not each GPCR candidate by using lentiviral gene transfer vectors. HIV infectability was measured in both cell lines after infection by replicative and non-replicative HIV-1 strains.

We identified 250 GPCRs co-expressed with CCR5 in circulating CD4 T lymphocytes. Among these GPCRs, we focused on the Epstein–Barr virus-induced gene 2 (EBI2). EBI2 is activated by 7α, 25-dihydroxycholesterol (7α,25HC) and plays a role in T cell-dependant antibody response and B cell migration.

We show that EBI2 interacts with CCR5 at the cell membrane and influences HIV behavior. EBI2 expression along with CCR5 reduces HIV entry and HIV infection when a single viral cycle is assessed. Surprisingly, EBI2 strongly augments HIV production over multiple replication cycles.

EBI2 dimerizes with CCR5 and influences HIV-1 replication in several ways.

Adeline GUIGUES, Sandrine GIMENEZ, Clément METTLING, Pierre CORBEAU and Vincent FRANCOIS.
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder caused by loss of motoneurons. ALS leads to atrophy and paralysis of striated muscles, which cause death within 3 to 5 years. Approximately 20% of familial ALS are caused by mutations in SOD1 gene. Mice overexpressing human SOD1 mutations develop a motor syndrome with features of human disease.

A chronic inflammatory response, associated with the accumulation of immune cells in the CNS, is a pathological feature of ALS. In the early phase of the disease, CD4+ lymphocytes invade the CNS and seem to negatively regulate the inflammatory response. However, the symptomatic phase is characterized by an increased infiltration of CD8+ T cells. The contribution of this cytotoxic cell population in the neurodegenerative process has been poorly investigated. Here, we propose to explore the impact of the infiltration of CD8+ T cells on the development of the disease.

Our results show that mutant SOD1G93A CD8+ lymphocytes, but not wildtype, trigger motoneurons death, specifically. Moreover, our co-culture experiments indicate that CD8-induced motoneuron death occurs in a contact dependent manner, via the recognition of the MHC-I complex exposed by motoneurons. Our results show that IFNg, Fas-Fas ligand and perforin-granzyme pathways are implicated in this neurotoxicity.

Beside this in vitro analysis, we performed a pharmaceutical depletion of CD8+ T cells, that permit to reduce by 70% the blood circulating CD8 T cells, and only by 50% the CNS-infiltrated ones. This peripheral depletion didn’t permit to improve neither the survival, the weight loss, nor the locomotor capacities of the SOD1G93A mice. This is consistent with the hypothesis that the cytotoxic effect of CD8+ T cells take place within the CNS. We will now use a genetic approach to deplete the entire CD8 compartment in SOD1G93A mouse, and asses the consequences on the pathophysiology of the mice.

Coque, E.1, Carrasco, G.E.2, Salsac, C.1, Vincent, T.1,3, Hernandez, J.2, Raoul, C.1. 1INSERM U1051 - Déficits sensoriels et moteurs, Montpellier, France. 2INSERM U1183, Cellules Souches, Plasticité Cellulaire, Médecine Régénératrice Et Immunothérapies Montpellier, France. 3Hopital Saint Eloi, Département d'immunologie, Montpellier, France.
Hetero-oligomerization of dopamine D1 and metabotropic glutamate mGlu5 receptors modulates cellular signaling

The dopamine (DA) neurotransmitter regulates important neural pathways by modulating glutamatergic transmission. Perturbations of DA signaling are implicated in the pathogenesis of many neuropsychiatric disorders including Parkinson’s disease (PD). Dopamine and glutamate signaling pathways converge on responsive neural populations such as the striatal medium-sized spiny neurons (MSNs). Because both glutamate and dopamine are required to induce and sustain MSNs plasticity, the particular molecular mechanisms involved at this synaptic triad are difficult to understand.

As already described for NMDARs-DARs, numerous observations suggest a postsynaptic interplay between mGlu receptors and DARs signaling pathways. Recent data, in the DA-denervated striatum, indeed suggest that mGluR5/PLC/PKC cascade is required to enable D1R agonist-induced ERK1/2 phosphorylation, hallmark of a molecular reorganization that is critical to the development of L-DOPA-induced dyskinesia.

Over the past few years, increasing evidence has suggested that G-protein coupled receptors (GPCRs) hetero-oligomerization can regulate receptor signaling through modulation of ligand binding, receptor delocalization or changes in G protein coupling. In the present work, we assessed the molecular mechanisms underlying a functional interplay between mGlu5 and D1 receptors, focusing on potential direct physical interaction between both receptors. We show in HEK 293 cells that mGlu5 and D1 can form hetero-oligomers involving their C-tails. As a functional consequence, mGlu5/D1R interaction seems to favour Gq/PLC/Ca2+ signaling pathway consistent with previous observations in the DA-denervated striatum. In the future, we will further investigate the rationale of dimerization-induced modulation of receptors signaling during synaptic plasticity in physiological and pathological conditions.

The human OPA1delTTAG mutation induces age-related auditory neuropathy in mouse

Mutations in the mitochondrial gene OPA1 leads to dominant optic atrophy that can be associated with hearing impairment. While the optic atrophy in OPA1 mutant is well characterized, the molecular and cellular mechanisms of the auditory deficit are still unknown. Here, we characterized the hearing dysfunction in OPA1-linked disorders in an Opa1 mouse model carrying the recurrent Opa1delTTAG mutation, which is found in 30% of all patients with dominant optic atrophy. The cochlear functions were investigated using auditory brainstem responses (ABRs), cochlear and compound action potential (CAP), endocochlear potential (EP) and distortion-product otoacoustic emissions (DPOAEs). Hyperacusis and tinnitus was assessed using the acoustic startle response (prepulse and GAP detection inhibition). The numbers of synapses and hair cells were counted using confocal microscopy, and scanning electron microscopy (SEM), respectively. Opa1delTTAG mice show progressive deafness, reminiscent of age-related hearing loss, as probed by the reduction in auditory brainstem responses and electrocochleography. In contrast, the DPOAEs and EP did not change. Together with the loss of inner hair cells ribbon synapses assessed in confocal microscopy, a disorganized myelin sheath with the degeneration of spiral ganglion neurons was observed in electron microscopy. Behavioral task suggest that Opa1delTTAG mice may exhibit hyperacusis. At the molecular level, we found out autophagy, mitophagy and mitochondrial supercomplex instability occurs in the spiral ganglion neurons that precede the neural degeneration. Altogether, these results will enable to design therapies for the auditory impairment in OPA1-related optic atrophy.

Carolanne Coyat\textsuperscript{1,2}, Hideaki Ogita\textsuperscript{1,2}, Emmanuelle Sarzi\textsuperscript{1,2}, Guy Lenaers\textsuperscript{1,2}, Jean-Luc Puel\textsuperscript{1,2} and Jing Wang\textsuperscript{1,2}. \textsuperscript{1}INSERM - UMR 1051, Institut des Neurosciences de Montpellier, 34295 Montpellier, France. \textsuperscript{2}Université Montpellier, 34295 Montpellier, France.
N°58 – Benoit GIRARD
Tackling epileptogenesis via the mGlu7 glutamate receptor

The development of spontaneous seizures remains a challenge in the treatment of epilepsy. While the molecular and cellular determinants of seizures and epilepsy have been described, only sporadic progress has been made when attempting to develop anti-epileptogenesis approaches. An increased glutamatergic activity is a hallmark of epilepsy, bearing devastating effects. The metabotropic glutamate receptor mGlu7 inhibits glutamate release from the presynaptic terminals, representing a beneficial feedback mechanism. Mutations of the GRM7 gene have been associated to a range of human pathologies, and GRM7 gene inactivation or mutation in rodents cause spontaneous epileptic seizures. Yet, the role of mGlu7 in seizure progression and its potential as a therapeutic target have not been explored, due to the paucity of pharmacological tools available.

Here we investigated the effect of mGlu7 in a model of epileptogenesis induced by repetitive injection of the convulsant pentylenetetrazole (PTZ kindling). We combined the use of new mGlu7-targeting compounds and genetic mouse models bearing mutation or invalidation of the GRM7 gene. mGlu7 mutant mice show a lower threshold for seizure progression, whereas wild-type mice treated with the mGlu7/4 agonist LSP2-9166 present a slower development of PTZ-induced seizures. Targeting mGlu7 has an impact neuronal damage and associated neuro-inflammation. Specific modulation of the mGlu7 receptor could represent a novel therapeutic approach to reduce pathological network remodelling.

Benoit Girard1.2.3, Delphine Rigault4, Francine Acher4, Julie Perroy1.2.3, Laurent Fagni1.2.3, Nicola Marchi1.2.3, Federica Bertaso1.2.3, 1CNRS, UMR-5203, Institut de Génomique Fonctionnelle, Montpellier, 34094, France. 2INSERM, U1191, Montpellier, 34094, France. 3Universités de Montpellier, UMR-5203, Montpellier, 34094, France. 4Université Paris Descartes, CNRS UMR-8601, Paris, 75006, France.
The serotonin 5-HT2A receptor is the primary target of psychedelic hallucinogens such as LSD, mescaline and psilocybin (agonists), which reproduce some of the core symptoms of schizophrenia and of second-generation antipsychotics such as clozapine, olanzapine and risperidone (antagonists or inverse agonists). Recent findings demonstrate that 5-HT2A receptors form heteromers with metabotropic glutamate mGlu2 receptors, another target of last-generation antipsychotics (agonists or positive allosteric modulators). The association of both receptors has profound consequences on their pharmacology and signal transduction properties as well as on the behavioural effects of drugs that bind to either 5-HT2A receptors or mGlu2 receptors. For instance, 5-HT2A receptor/mGlu2 heteromer formation is essential for the expression of psychotropic-like effects of hallucinogens and imbalanced activity and coupling properties of 5-HT2A and mGlu2 receptors within the heterocomplex might be one of the molecular substrates for a susceptibility to schizophrenia. To get further insight into the mechanism of action of drugs acting at 5-HT2A/mGlu2 heteromers, we explored their impact upon the phosphorylation pattern of each receptor by high-resolution mass spectrometry. We show that hallucinogenic 5-HT2A receptor agonists (LSD, DOI) but not non-hallucinogenic 5-HT2A receptor agonists promote 5-HT2A receptor phosphorylation at Ser280 located in the i3 loop, a region important for receptor desensitization, both in HEK-293 cells and in mice prefrontal cortex. Correspondingly, Ser280 phosphorylation was responsible for the lower capacity of hallucinogens to promote receptor desensitization and internalization, compared with non-hallucinogenic agonists. Conversely, several phosphorylated residues were identified in the C-terminal domain of mGlu2 receptors co-expressed with 5-HT2A receptors in HEK-293 cells. Glutamate or LY379268 (orthosteric mGlu2 agonist) treatment increased the phosphorylation state of some of these residues, an effect prevented by the co-application of the synthetic hallucinogen DOI, which alone did not affect mGlu2 phosphorylation. Collectively, these findings reveal novel molecular substrates that might underlie the behavioural effects of drugs acting at each subunit of 5-HT2A/mGlu2 heteromers.

Samy Murat1,2, Samah Karaki1,2, Carine Becamel1,2, Clotilde Mannoury la Cour3, Mark J. Millan4, Philippe Rondard1,2, Laurent Prézeau1,2, Joël Bockaert1,2, Philippe Marin1,2,*, Franck Vandermoere1,2,*. 1CNRS, UMR-5203, Institut de Génomique Fonctionnelle, Montpellier. 2INSERM, U1191, Montpellier. 3Université Montpellier, F-34094 Montpellier, France. 4Institut de Recherches Servier, Croissy-sur-Seine, France. *These authors equally contributed to the study.
Physiological pain is essential for individual survival, but chronic pains are purely deleterious for the organism and the life quality. Unfortunately, current therapies are limited to drugs with a low efficacy or with a bad benefit/risk ratio. It is thus urgently necessary to better understand the establishment and persistence mechanisms of neuropathic pain in order to design efficient therapeutic strategies against this pathology. Many studies have shown that T-type calcium channels are involved in chronic pain states, like Cav3.2 subtypes present all along the pain circuit. In the peripheral nervous system, Cav3.2 channels have pronociceptive impact and are now approved as a target for innovative therapies. The Cav3.2 channel role at superior level, and especially in the spinal cord, a crucial hotspot of nociceptive information convergence, integration and transmission, remains to be found.

Thanks to a Cav3.2-GFP-Lox murine model created by the team, we were able to i/ identify and precisely localize Cav3.2 positive neurons in all the nervous system and ii/ induce tissue specific deletion of Cav3.2 by the Cre recombinase action, to evaluate effects on pain sensitivity. We found that Cav3.2 is mainly expressed in excitatory, but also in inhibitory spinal neurons. Knocking-out spinal Cav3.2 by a viral approach has demonstrated i) an abolition of cold and mechanical allodynia like behaviors under neuropathic conditions in males and females and ii) an alteration of the hot perception, under pathological pain conditions, with a differential effect in a sex dependent manner.

The Cav3.2 channel deletion has thus preventive effects on the two main symptoms of neuropathic pains. Clinical perspectives of T-type calcium channel antagonists are accentuate by these results, as they showed the utility to target spinal Cav3.2 additionally to the peripheral effects already known.

FRUQUIERE Antoine, LAFFRAY Sophie, BOURINET Emmanuel. Equipe "Canaux calciques et nociception", Institut de Génomique Fonctionnelle, UMR 5203 CNRS, U1191 Inserm, Univ Montpellier.
Background and Introduction: In our aging society, age-related hearing loss or presbycusis is increasingly important. Based on observations of temporal bones from patients with presbycusis, Schuknecht (Schuknecht and Gacek, 1993) proposed the classification into three major forms, namely sensory, neural, and strial presbycusis according to the location of damage (sensory epithelium, spiral ganglion neuron, or stria vascularis). To date, the mechanisms underlying the age-related hearing loss remain unclear. Based on our previous study (Menardo et al., 2013) showing that the premature age-related hearing loss observed in senescence-accelerated mouse prone 8 (SAMP8) mice was correlated with altered levels of antioxidant enzymes and decreased activity of mitochondrial functions, we hypothesized that the oxidative stress may play a key role in presbycusis.

Methods: To investigate the contribution of the oxidative stress in presbycusis, we exposed the p3 mouse cochlear explants to hydrogen peroxide (H2O2) in vitro. The cochlear cell senescence or degeneration was evaluated using the specific biomarkers. In addition, the role of endogenously-produced ROS in age-related hearing loss was assessed in adult p66KO mice which have a decreased ROS production.

Results: Our results provide the evidence that the oxidative stress plays a key role in age-related hearing loss and cochlear sensory hair cell apoptosis. We demonstrate that H2O2 exposure induced a premature occurrence of cochlear sensory hair cell senescence and apoptosis, illustrated by the massive increase of the cell senescence and apoptosis biomarkers such as SA-beta gal, gH2AX, Annexin V and TUNEL, mainly in the cochlear sensory hair cells, but not in the spiral ganglion neurons. Interestingly, our in vivo results from p66 KO and WT mice provided the functional and morphological evidence that the targeting of oxidative stress by genetic interventions protect the cochleae against age-related sensory hair cell death and hearing loss.

1INSERM - UMR 1051, Institut des Neurosciences de Montpellier, 34295 Montpellier, France. 2Université Montpellier, 34295 Montpellier, France.
TRIM proteins regulate ubiquitination and degradation of the anti-apoptotic protein Bfl-1

Bfl-1 is an anti-apoptotic protein of the Bcl-2 family which is often overexpressed in poor prognosis lymphoma and chemoresistant melanoma for which no efficient treatment exists. Previous studies have shown that Bfl-1 is a short-lived protein, whose level is tightly controlled by ubiquitination. Here, we show that polyubiquitination and proteasomal degradation of Bfl-1 are regulated by the E3 ubiquitin-ligases TRIM28 and TRIM17. Indeed, TRIM28 co-immunoprecipitated with Bfl-1. Moreover, TRIM28 overexpression strongly destabilized Bfl-1 and increased Bfl-1 ubiquitination level in cells whereas TRIM28 silencing reduced it. From another side, the expression of TRIM17 stabilized Bfl-1 and decreased its ubiquitination. Interestingly, coexpression of TRIM17 disrupted the Bfl-1/TRIM28 interaction and strongly decreased TRIM28-mediated Bfl-1 ubiquitination.

Taken together, these results identify TRIM28 as the first E3 ubiquitine ligase of Bfl-1. Moreover, TRIM28-mediated degradation of Bfl-1 appears to be regulated by TRIM17 possibly by disrupting the interaction between TRIM28 and Bfl-1.

Our future work will explore these mechanisms in pathological models, in order to open new avenue to promote Bfl-1 degradation and thus reactivate apoptosis in tumors that rely on Bfl-1 overexpression.

Loïc Lionnard, Barbara Mojsa, Francesca Guardia, Abdel Aouacheria, Stéphan Mora, Iréna Lassot, Solange Desagher and Jérôme Kucharczak. 1 Institut de Génétique Moléculaire de Montpellier, IGMM UMR 5535 CNRS, 1919 route de Mende, 34293 Montpellier cedex 5, France. 2 Laboratoire de Biologie Moléculaire de la Cellule, Faculté de Médecine Lyon-Sud, LBMC UMR 5239 CNRS - UCBL - ENS Lyon - HCL, 46 Allée d'Italie, F-69364 Lyon Cedex 07, France.
Colorectal cancer (CRC) is a disease affecting from the epithelial cells lining the colon or rectum. It is the third most common type of cancer worldwide. Treatments used for colorectal cancer may include some combination of surgery, radiation therapy and chemotherapy. Once metastasis has occurred, the patients 5-year survival falls dramatically. Therefore, it is important to define the mechanism leading to development and metastasis spreading of CRC in order to find more efficient therapies. During tumor progression, cancer cells can undergo an Epithelial to Mesenchymal Transition (EMT) associated with the acquisition of invasive and stem cell properties. Cyclin A2 is known as a key regulator in cell proliferation. Interestingly, Jean Marie Blanchard’s lab found a novel function of CyclinA2 in regulating cell invasion and EMT. They also found that CyclinA2 expression is increased in primary tumor, but decreased in hepatic metastasis in CRC patients. In order to study the in vivo relevance of CyclinA2 in EMT and metastasis in colorectal cancer, I will use two mouse models: the experimental metastasis mouse model and the Azoxymethane/Dextran Sulphate Sodium (AOM/DSS) model. In the former mouse model, I will check the impact of Cyclin A2 inactivation on metastases spreading by cecal injection of CRC cell lines bearing an inducible shCyclinA2. For the second model, mice will be exposed to an AOM/DSS treatment that results in the development of colitis associated carcinogenesis in 60 days. In this model, I will study cyclin A2 fl/fl ERT2 Villin–Cre mice, where the expression of Cre under the Villin promoter can be induced by tamoxifen treatment, and induce cyclin A2 inactivation at different stages of carcinogenesis.

Yuchen GUO, Benedicte LEMMERS, Michael HAHNE.
N°64 (with Flash Talk) – Laura YAZDANI
Translational control in cancer cells

One of today’s biology challenges is to comprehend how protein expression is regulated with such accuracy at sub-cellular level. From embryonic development to cell differentiation and metabolism, translation is used to fine-tune protein levels in both time and space. Mutations that affect translation machinery lead to a wide variety of diseases including cancer. This project aim to establish a causal relationship between translational control and colorectal cancer progression and decipher the molecular mechanisms involved in this process. Nothing is known about translational control in cancer stem cells (CSC), though these cells represent a perfect model to test whether the ribosome takes an active part in the “stemness” balance driving tumor adaptation/progression.

We defined 2 goals:
1) Study translational control in CSC and non-CSC by comparing transcriptome and translatome from both CSCs and non-CSCs on a colon carcinoma cell line
2) Identify and characterize deregulated RPs in CSC.
Diffuse low grade gliomas (DLGG) are primary tumors that affect functional regions in the adult brain, even though their proliferation rate is slow, they can degenerate into a higher and more aggressive state after resection. The difficulty resides with the fact that these tumors are characterized by a heterogeneous non-predictable histological profile. The majority of the DLGG have a non-sense mutation for the IDH1 gene that will lead to the formation of a new oncometabolite and thus a hypermethylation of the genome and by consequence to the blockage of the cell differentiation within the tumor. The characterization of the cancerous cells and the comprehension of the signaling pathways evolved in the cancerous progression, plus the identity of the microenvironment are important and essential in order to understand these tumors. It is known that cancerous tissue consists of a heterogeneous bulk and contain a non-tumoral stroma with tight interactions with the mutated cells. This ecosystem seems to be a regulating complex of the dormancy and the cancerous proliferation, such as the BMP, Wnt and Mapk pathways. Additionally, until now there is no in vitro model of these tumors due to the low proliferation rate; the establishment of a new cell line with the IDH1 mutation is conducted with different viruses targeting the studied signaling pathways and some effectors of the cell cycle leading hopefully to a cellular proliferation without changing the IDH1 mutated cells profiles.

Gene expression is tightly controlled to ensure a wide variety of cell types and functions. The development of diseases, particularly cancers, is invariably related to deregulations of these controls. Our project aims to model the link between RNA expression and DNA features in regulatory regions (typically, presence/absence of transcription factor motifs [1] in promoters).

Several studies have shown that penalized linear regression (LASSO) is suitable for this problem, that requires selecting variables in high dimensional data [2]. Using similar approach, we were able to model gene expression in several cancers. Further investigations showed that the inferred model is not equally efficient for all genes. More precisely, the model only fits a certain class of genes with specific DNA features. This is likely due to the lack of suitable predictive variables for all other genes.

Our perspective is now to extend this model with other types of genomic variables, and to design a clustering approach to identify coregulated genes and train a specific regression model for each cluster.


Chloé BESSIERE\textsuperscript{1,2}, May TAHA\textsuperscript{1,2,3}, Florent PETITPREZ\textsuperscript{1,2}, Charles LECELLIER\textsuperscript{1,2}, Laurent BREHELIN\textsuperscript{2,4}, Sophie LEBRE\textsuperscript{2,3}. \textsuperscript{1}\textsc{IGMM}, \textsuperscript{2}\textsc{IBC}, \textsuperscript{3}\textsc{IMAG}, \textsuperscript{4}\textsc{LIRMM}. 
N°67 – Hanane MANSOURI
Combinatorial strategy targeting the tumor microenvironment of triple-negative breast cancer with anti-protease antibody

New treatments are required for triple-negative and hormono-resistant breast cancers. In breast cancer, the aspartic protease cathepsin D (Cath-D) is an independent marker of poor prognosis. Cath-D is overproduced by breast cancer cells and the pro-enzyme is hyper-secreted into the tumor microenvironment. Liaudet-Coopman’s team has made major contributions to the understanding of oncogenic roles of cath-D released in excess by cancer cells in the extracellular space of breast cancer.

With the support of the LabEx MabImprove, Liaudet-Coopman’s team generated human monoclonal antibodies by phage display to target Cath-D secreted in the breast tumor microenvironment. Two scFv cloned into human IgG1 format (F1 and E2 IgG1) inhibited triple-negative and ER+ breast cancer cell wound healing, colony formation and three-dimensional outgrowth in Matrigel. Anti-Cath-D IgG1 F1 and E2 significantly reduced tumor growth of triple-negative MDA-MB-231 breast cancer cells in nude mice. These results suggest that antibody-based targeting of Cath-D may have therapeutic efficacy for breast cancer treatment.

The objective of my PhD thesis is to develop a combinatory strategy to target the tumor microenvironment of triple-negative breast cancers with anti-Cath-D antibodies. First, we will evaluate the expression of Cath-D in different subtypes of breast cancer in order to determine which patients could benefit from a treatment with anti-Cath-D antibodies. We will study the therapeutic effect of anti-Cath-D IgG alone or in combination with chemotherapy in several breast cancer models (PDX (patient-derived xenograft), mouse mammary cancer cells in syngenic mouse, transgenic mouse model of breast carcinogenesis). Finally we will assess the in vivo mechanisms of action of anti-Cath-D antibodies.

Dissection of E4F1 function on cell cycle checkpoints and DNA repair in response to genotoxic stress

Our recent transcriptomic and ChIPseq experiments had shown in mouse embryonic fibroblasts (MEFs) that the multifunctional protein E4F1, directly controls genes involved in mitochondria functions and cell-cycle checkpoints, including Chek1, a major component of the DNA damage response (genomic data). Coordination of these cellular functions by E4F1 appears essential for the survival of p53-deficient transformed cells. (Rodier et al., Houlès et al.). The aim of my PhD’s project is to determine the importance of E4F1 action in human cancers. For this purpose, I will focus on cell cycle checkpoints and DNA damage response in breast cancer cell lines. Based on these previous results we determined a degenerated consensus sequence of 9 nucleotides present in the promoter of E4F1 target genes in MEFs, and by an in silico study we established a list of the cancer related human genes that contains this sequence in their promoters. First, I will test these predicted targets by ChIP-qPCR in SUM 159 human breast cancer cells and then I will perform ChIPseq experiments in this cell line in order to identify new putative targets. We hope that these experiments, will allow us to determine the E4F1 cistrome in human breast cancer cell lines in response to genotoxic stresses.

A direct interaction between E4F1 and CHK1 was recently described (Grote et al.) and previous work in the lab have shown that this interaction is dependant on E4F1 phosphorylation on 4 different serines identified by mass spectrometry. I will determine if this interaction occurs on the chromatin and if it is necessary for E4F1 transcriptional activity on its target genes.

This project would allow us to identify the transcriptional program activated by E4F1 in human breast cancer cell lines in response to genotoxic stresses that are important for cancer cells survival. From this study, we hope to determine new potential therapeutic targets.

Kalil Batnini, Thibault Houles, Genevieve Rodier, Claude Sardet. 1Institut de Cancérologie de Montpellier, Montpellier, F-34090, France. INSERM, U1194, Montpellier, France. Université de Montpellier, F-34090, Montpellier, France.
The RIP140 protein is a transcriptional co-regulator which represses the activity of many transcription factors and is involved in various physiological processes. By combining the use of mouse models with molecular and cellular biology experiments, recent results from our laboratory have demonstrated that RIP140 controls intestinal homeostasis and could play a tumor suppressor role in colorectal cancer. Indeed, RIP140 regulates intestinal cell proliferation and differentiation by inhibiting the Wnt signaling pathway (Lapierre et al, JCI, 2014). Interestingly, both in intestinal homeostasis and tumorigenesis, a strong crosstalk exist between Wnt and Notch signaling pathways. For instance, in colon cancer cells, Notch signaling is a downstream target of β-catenin hyperactivation and the HES1 gene (the major effector of the Notch pathway in the intestine) is also regulated by the Wnt pathway. In order to define if and how RIP140 controls the Notch pathway in human colon cancer cells, we set up reporter assays, RT-qPCR and Western blot experiments using the HES1 gene as the main output of the Notch signaling. In reporter assays experiments, the modulation of the Notch pathway (by NICD ectopic expression or using Notch inhibitors) revealed that RIP140 exerts a positive NICD-dependent effect on HES1 expression. In RT-qPCR and Western blot experiments, this positive effect of RIP140 was confirmed and shown to be dose-dependent, suggesting that RIP140, depending on its expression level, could either positively or negatively regulates HES1 expression. In addition, the use of a Wnt activator (LiCL) has shown that RIP140 is involved in the crosstalk between these two signaling pathways. Further experiments such as ChIP and coimmunoprecipitation assays are needed to clarify the molecular mechanisms by which RIP140 regulates HES1 gene expression. The effects of RIP140 on cellular parameters such as cell proliferation or differenciation will also be investigated in vitro or in vivo using transgenic mouse models.

Keywords: Colon cancer, Notch and Wnt pathways, HES1 gene, RIP140.

Nour SFEIR, Sandrine BONNET, Marion LAPIERRE, Vincent CAVAILLES.
Integrated models for assessment of significant biological mechanisms in oncopharmacology and aging: C. elegans and H. dujardini

We work on two integrated models for assessment of significant biological mechanisms in oncopharmacology and aging: Caenorhabditis elegans and Hypsibius dujardini. Caenorhabditis elegans is a small organism composed of 959 cells at the adult stage. Adult hermaphrodite is 1 mm length and 60 μm diameter. C. elegans is a suitable laboratory model that allows physiological impact evaluation upon exposure to exogenous molecules.

We have developed new molecular and genetic tolls that allow us to look at the aging process control in relation with various treatments in order to find unidentified targets. For example, Chicoric acid can act on muscle mitochondria homeostasis as well as on metabolism linked with type 2 diabetes. We have started to analyse C. elegans aging impact of Chicoric acid and found an unexpected positive effect even at unexpected very low (micromolar) concentrations.

We have also tested some gas in term of toxicity, development, fertility and mutagenicity for an industrial toxicity assessment. In the same way, we have studied the anti-aging effect of several waters.

Our quantitative C.elegans technology can include liquid, gas, soluble or insoluble drug screenings on identified or unidentified molecular targets (or pathways) as well as monitoring of mitochondrial, metabolism or aging impact as well.

Hypsibius dujardini hold exceptional characteristics that enable it to withstand high pressure, space vacuum, radiation, dehydration,… This tardigrade can adopt a resistance state when conditions become harsh.

We have adapted new techniques for living tardigrade to uncover mitochondria adaptation and control during metabolic activity shutdown of the anhydrobiotic state.

We have measured the O2 consumption in the basal state and during anhydrobiotic state exit.

We have also tested several mitotracker in order to address questions about mitochondria control level on anhydrobiosis entry and recovery."ATR signaling complex. Thus, UBA1 inhibitors could be used to target ATR signaling in cancer cells.

Organizing Committee

The CBS2 Day 2016 is the 14th edition of CBS2 Days. This event was organized by the CBS2 Association, with the support of the CBS2 Doctoral School and BioCampus Montpellier.

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The organizing board wants to thank particularly the researchers who accepted to be the Jury of this CBS2 Day 2016.

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