INSTITUTE OF HUMAN GENETICS

Scientific Report

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INSTITUTE OF HUMAN GENETICS

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I G H

INSTITUTE OF HUMAN GENETIC

PHILIPPE PASERO

Associate Director

The Institute of Human Genetics (Institut de Génétique Humaine, IGH) is a CNRS unit located in the fast-growing Arnaud de Villeneuve biomedical campus of Montpellier, which includes several CNRS and INSERM laboratories (Centre de Biochimie Structurale (CBS), Institut de Génomique Fonctionnelle (IGF), etc.), the future University of Montpellier School of Medicine (University of Montpellier 1) and academic hospitals. It is adjacent to the site of the University of Montpellier and close the Center for Cancer Research (ICM). The Institute occupies a surface of 3800 m2.

IGH hosts around 200 staff and student researchers, working in 20 research groups and including scientists from CNRS, INSERM, University and Hospital researchers, engineers, technical and administrative staff (37), post-doctoral fellows (41), graduate students (22), undergraduate students and visiting scientists.

The IGH aims at providing a first-class scientific environment for the development of innovative research projects. The excellence of the research carried out at the IGH is attested by the quality of the scientific production, the number of awards attributed to scientists working at the Institute as well as the prestigious grants that support their research, particularly three grants from the European Research Council (ERC).

Currently, the IGH houses 20 research groups distributed in the three scientific departments (Genome Dynamics, Genetics & Development and Molecular Bases of Human Diseases).

Director's foreword

The "Institut de Génétique Humaine" (IGH) is a high-profile institute devoted to basic biomedical research. Throughout its 15 years of life, it has provided an excellent environment in which it is possible to carry out innovative, frontier-breaking science and where the quality of the technical facilities, infrastructure and administrative department matches and supports the high scientific output of the IGH.

IGH scientific life

The IGH is characterized by a vibrant day-to-day activity that boasts both scientific and extra-scientific events which contribute to the exciting science and the pleasant daily atmosphere of the Institute. Furthermore, routine events are complemented by special meetings every year. The main events that characterize the IGH community life are:

- Weekly «external» seminars given by invited scientists. Most of these seminars are given by internationally-renowned researchers and all are held in English;
- The annual IGH Seminar Series in which leading are invited by the Institute's departments to give keynote talks on their research work.
- Weekly «internal» seminars where scientists, post-doctoral fellows and PhD students expose their results and research projects. The lively informal discussions characterizing these seminars are continued in a friendly atmosphere during the Pizza time after the seminars;
- Scientific retreats (every second year) organized by each Department in order to facilitate scientific interaction in beautiful places free from the everyday worries of laboratory life;
- The IGH Retreat, a meeting that brings together all the Institute staff every second year, alternating with the department retreats;
- The ITA workshop, where PIs or researchers present the research done at IGH with a lay public language and entertainment for the administrative, technical and engineering staff is provided
- PhD student and postdoc workshops held every year, where data are presented and discussed in a dynamic and informal manner
- Organization of various high-level meetings like the biennial EMBO conference series on Nuclear Structure and Dynamics and others. For an exhaustive list, see http://www.igh.cnrs.fr/EN/seminaire.php#
- IGH researchers are frequently involved in the organization of practical courses (Ateliers INSERM and others) to train scientists in specific approaches on which they have high-profile expertise.

Teaching activities

The IGH is strongly involved in teaching and has a close relationship with the University of Montpellier. Several Professors and Associate Professors carry out their research activities at the IGH. IGH is an active member of the Doctoral School «Biology and Health» (CBS2) of the University of Montpellier. Every year, 25-30 graduate students are pursuing their PhD program at the Institute, and 8-10 of them defend their thesis. In addition, about 20 Master students do their practical laboratory training at the IGH each year.

Technical facilities

The IGH offers an excellent technical environment and all the infrastructures needed to carry out cutting-edge molecular, cellular and developmental biology research. It also possesses two biosafety L3 laboratories. One of the main strengths of the Institute is its capacity to react rapidly to the need of updating its facilities in response to the fast technological progress of science. For the last three years we have been running an «Agence de Biomédecine»-certified laboratory devoted to the study of human embryonic stem cells. In 2009, we opened a state-of-the-art 100 m2 imaging facility.

This facility, called MRI – IGH, has imaging equipment which is worth more than 3 million Euros, with more than 10 top-level epifluorescence microscopes, 2 confocal microscopes, including a Leica SP8 confocal microscope equipped with a UV laser that allows studies with photoactivatable GFP and the generation of directed UV damage, and the "OMX" super-resolution fluorescence microscope. This puts our imaging facility at the forefront in fluorescence imaging acquisition/analysis in France and Europe. The IGH has also equipped the «Montpellier GenomiX» genomic facility with an Illumina HiSeq 2000 instrument, which joins the newly acquired Illumina HiSeq 2500 instrument and microarray equipment. Together with their bioinformatic analysis pipeline, these instruments allow high throughput genomic analyses. This facility is installed in the new building of the Institute of Functional Genomics (IGF) that communicates directly with the IGH. The Institute also has rodent, Drosophila and Xenopus facilities.

Finally, the IGH is a member of "Biocampus", the CNRS-funded servicing unit that provides easy access to all technical facilities available in the city to the whole Montpellier research community. The facilities located at the IGH (particularly the animal house and the imaging facility) are thus available to the whole scientific community of Montpellier.

Institute Governance

The acting director, Giacomo Cavalli, and the deputy director, Philippe Pasero, took up their functions in January 2011. They were assisted by a steering committee, composed by the department heads (Martine Simonelig for Genetics and Development, Bernard de Massy for Genome Dynamics, Monsef Benkirane for Molecular Bases of Human Diseases and Marcel Méchali, head of the upcoming Genopolys). Scientific issues are discussed within the group leader board and they are further examined, along with budget and other policy issues, by the 15-member Institute Council, composed by the directors and a mix of nominated and elected members from all the personnel bodies: researchers, post-doctoral fellows, PhD students, engineers, technicians and administrative managers.

Starting from 2011 the IGH Scientific Advisory Board (SAB) started its activity. The SAB includes Hervé Chneiweiss, University Paris Descartes, Paris, France; Denis Duboule, University of Geneva, Switzerland; Edith Heard, Institut Curie, Paris, France; Stéphane Noselli, Institute of Developmental Biology and Cancer, Nice, France; and Didier Trono, from the Ecole Polytechnique Fédérale de Lausanne, who chairs the SAB. The SAB members cover the research fields of the three IGH departments and examine the overall Institute activity every two years. The SAB chairman, Didier Trono, also took part in the laboratory evaluation by the AERES, held from February 5 to 7, 2014. The SAB fosters scientific creativity and the quality of IGH management by giving advice on Junior group performance, new hiring and other scientific policies.

A year of exciting science

Last year's scientific achievements have been again impressive! It would be too long to discuss all the main findings published by the IGH groups but it is remarkable to see how several laboratories have published striking discoveries. We are particularly delighted to note that, following external peer-review evaluation as well as AERES scrutiny, two junior IGH labs, Domenico Maiorano and Rosemary Kiernan, have obtained tenure. We wish them the best of times in the coming years as senior IGH members

IGH and the initiative "investissements d'avenir" (investments for the future) of the French Ministry of Research

To increase French scientific competitiveness, the French Ministry of Research launched three years ago a large investment campaign in order to fund various research-related components, such as acquisition of large equipment, large facilities and infrastructures, Centers of excellence and Campuses of excellence.

The IGH PI Marcel Méchali is coordinating a Center of Excellence (Labex) called EpiGenMed: From Genome and Epigenome to Molecular Medicine. In total, 49 internationally renowned research laboratories working in different fields (mathematics, biophysics and biochemistry, molecular, cellular and developmental biology, cancer biology, infectiology and neurobiology) joined forces to address the following main questions:

- How do genome and epigenome regulations impact on cell proliferation, differentiation and development?

- What are the interactions between host and infectious pathogens, how do they induce diseases and how can we use this knowledge to cure the world's most critical infectious diseases?

- What are the molecular bases of the cell signaling processes in the central nervous system and in the sensory organs and how do signaling dysfunctions induce neurological, neurodegenerative and sensory disorders?

The project will run until 2021 thanks to massive funding that will serve to support PhD and post-doctoral fellowships, group leader hires, research, teaching and scientific communication activities as well as the clinical exploitation of the results. The third round of the international PhD and Postdoc programs that were run in 2014 have been heavily subscribed by excellent applications. IGH researchers are heavily involved in the EpiGenMed research programs and they coordinate 3 of the 5 programs (biophysics and systems biology; epigenetics and genome dynamics; cell cycle, cell fate and development; infectious disease and immunology; cell signaling and neurobiology). Thus, IGH is a major steering force of this innovative large-scale project.

IGH, Genopolys and the Rabelais cluster for biology and health

Under the auspices of the University of Montpellier, the CNRS and the INSERM as well as of the other major science institutions of the region, the Rabelais cluster for biology and health (http://www.polebiosante-rabelais.fr/) has begun its activity in 2013 in order to coordinate regional scientific policies in the health and biology fields, concerning teaching, research, scientific communication and technology transfer. The cluster was visited in November 2013 by its SAB, who formulated an enthusiastic appreciation of the quality of biomedical sciences in the city. In this occasion, the cluster opened its scientific animation activity with an international symposium that was held at the anatomy amphitheater of the Faculty of Medicine, a very successful event that gathered over 200 scientists, medical doctors and industry leaders from the region and beyond. The IGH is represented in the steering board of the Rabelais cluster by Giacomo Cavalli, coordinator of the epigenetics research program, and by Marcel Méchali, head of Genopolys. In 2014, the Rabelais biomedical cluster organized several thematic meetings hosting local and international speakers. Furthermore, an innovative prize for your talents was launched, granting two prizes in each research program. 4 of the 12 prizes were won by young IGH scientists, highlighting once more the exceptional level of our research.

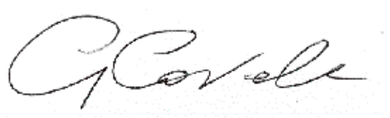
Genopolys (www.genopolys.fr), a unit led by Marcel Méchali and dedicated to scientific training and outreach initiatives and to science communication, opened in June 2013 and started its activities. To celebrate a strong beginning, featuring the participation in the "Fête de la science" and other exciting outreach initiatives, a symposium was held in December 2013. The unit, which was formerly part of IGH, acquired independence this year. A large number of activities directed to citizens, students, scientists, medical doctors and entrepreneurs has been carried out throughout the year. The IGH wishes a fabulous continuation of independent activity, while maintaining its strong commitment to help its establishment and to provide idea for events.

All the best for 2015!

IGH has achieved strong scientific goals and has improved its organization during the last year. The independent external evaluation by the AERES agency has confirmed that IGH is a world-class institute. Just one example of its international visibility is given by the Webometrics ranking of research institutions, that places IGH stably among the best French scientific Institutes in the world arena (see http://research.webometrics.info/en/Europe/France%20?page=1). As always, we are committed to further enhance the quality and impact of our science, while maintaining a friendly and easy-going atmosphere. It is thus my pleasure to wish a great year to come to all IGH members.

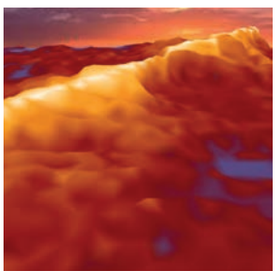
2015 will mark an important transition since, continuing the tradition by which Institute directors are turning over at the end of each evaluation period, Giacomo Cavalli and Philippe Pasero will pass the lead to the new director Monsef Benkirane and his deputy director Dominique Giorgi. We wish to congratulate them warmly for accepting to serve the IGH community for the next mandate and wish them a spectacular time, full of achievements and discoveries.

Enjoy the future!



Department of Genome Dynamics

Director : Bernard de Massy



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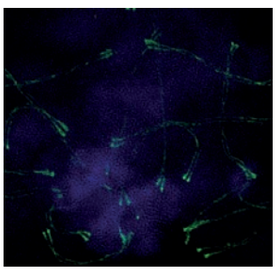

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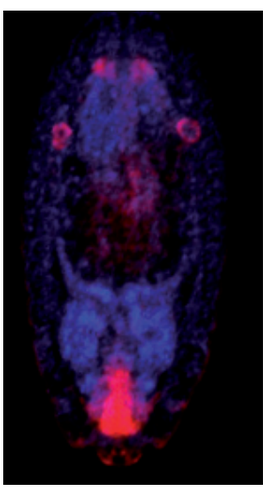
Corinne Grey



Autorship :
Abdou Akkouche

The department of Genome Dynamics includes groups focusing their research on understanding the multiple functions of the genome by analyzing different aspects of its biology in various model systems (Drosophila melanogaster, Xenopus leavis, Caenorhabditis elegans, Mus musculus and human cells). These aspects which are often both genetically and epigenetically controlled include DNA replication, recombination, activity of mobile elements, transcription, RNA splicing and chromatin structure and dynamics.

Research on DNA replication aims at identifying origins of replication, understanding the molecular mechanisms of origin firing and how these events are regulated in order to take place at the right time and only once per cell cycle. A special form of the cell cycle is the meiotic division that generates gametes, and our department is exploring the processes that ensure the proper hereditary transmission of the genome by studying the mechanisms of recombination and chromosome segregation during meiosis. Specific projects are focused on understanding the mechanism of the programmed induction of DNA double strand breaks during meiosis and their impact on genome stability and evolution. How genome integrity is maintained in the germline, particularly via the control of the activity of mobile elements, is also addressed through the analysis of the regulation of a small RNA family called piRNAs.

Several projects also aim to determine how the organization of the genome, at the level of chromosomes and chromatin, impacts on its activities including the ones mentioned above. Pioneering approaches are developed to analyze regulations taking place at different levels within the nuclear space, from the recently identified topological domain organization of chromosomes to chromatin and histones. Specifically, we aim at understanding how the closed, compact chromatin structure called heterochromatin is regulated and its biological relevance for development and genome stability in regions of the genome, such as telomeres, peri-centromeres and ribosomal DNA. How local chromatin modifications and the three-dimensional organization of chromosomes in the nucleus are integrated and how they impact on gene expression is also addressed through the study of the Polycomb and Trithorax protein families. At the gene level, factors that are involved in gene expression, and thus controlling cell identity and differentiation are investigated. In particular the important regulations mediated by RNA polymerase pausing, by alternative splicing and by non coding RNAs are explored through search of new components and coordination between these processes and the structure and organization of the chromatin.

Our department has a strong expertise in a variety of approaches, particularly in biochemistry, genetics and molecular and cellular biology. State-of-the-art microscopy, imaging, chromatin and chromosome organization with bio-informatic analyses of next-generation sequencing data have also been recently developed by several groups. The department research groups are engaged in several collaborations that are fueled by common interests, by sharing and developing novel technologies, within an excellent scientific atmosphere and by formal laboratory interactions, such as the department retreats. In addition to the interactions within the department, several of our teams collaborate with laboratories in the two other departments of the Institute to understand how genome regulation drives development and its relationship with human pathologies.

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Chromatin and Cell Biology

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We are more than our DNA! In the last couple of decades it has become clear that chromosomal components such as histones, regulatory proteins and noncoding RNAs contribute to regulate all aspects of DNA function and contribute to heredity. Our lab has mainly focused on the analysis of proteins of the Polycomb and Trithorax groups: key regulators of the expression of major developmental genes that coordinate the processes of cell differentiation and cell proliferation. Polycomb proteins are able to silence gene expression, while Trithorax proteins counteract gene silencing in the appropriate cells. We have studied how Polycomb and Trithorax proteins are recruited to DNA, we published the first large-scale mapping of the distribution of Polycomb group proteins along Drosophila chromosomes and we demonstrated that polyhomeotic, a Polycomb group gene, is a tumor suppressor that controls cell proliferation by regulating Notch signaling.

A distinctive feature of these proteins is their ability to maintain the memory of gene regulatory states through successive mitotic divisions in the different cell lineages. We showed that the regulation of chromosome architecture by these proteins contributes the transgenerational epigenetic inheritance of chromatin states by revealing that the transmission of this mitotic and meiotic cellular memory can bring into play long-distance chromosomal interactions in the three-dimensional space of the cell nucleus. We then extended the analysis of chromosome architecture by analyzing at genome-wide scale the contacts made by each locus with all other chromosome loci in the genome. From this study, we deduced the principles governing chromosome organization and the functional implications of regulation of genome architecture. We further analyzed the nuclear organization of Polycomb proteins by performing a genome-wide screen for components regulating the 3D distribution of these proteins in the nucleus and identified SUMO components as important regulators that affect Polycomb function during development.

We also identified an exquisitely specific function of the PRC2 polycomb complex in the determination of the Drosophila oocyte. Inactivation of PRC2 genes results in the loss of silencing of cell cycle genes, which induces a fate switch whereby the oocyte transdetermines into a nurse-like cell. This research has thus uncovered a new, critically important function for Polycomb proteins in the transmission of life to subsequent generations.

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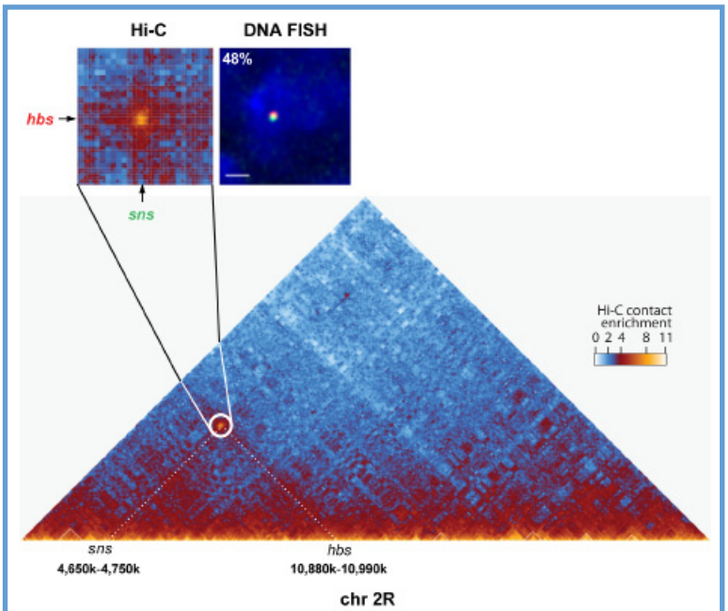


Figure 1. Hi-C, a molecular biology method to map interactions between chromatins sequences in vivo, is used to explore the spatial organization of the genome in Drosophila embryos. A cluster of strong interactions between two myoblast-specific genes, hbs and sns, located ~6 Mb apart on one chromosome arm, is highlighted. This interaction was also shown to occur at high frequency by DNA FISH (fluorescent in situ hybridization).

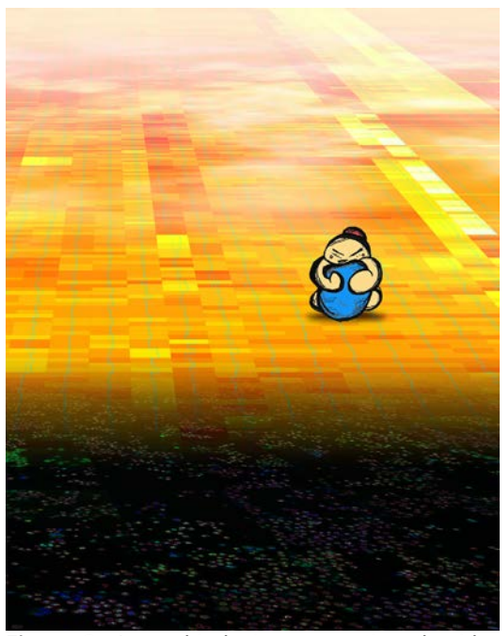
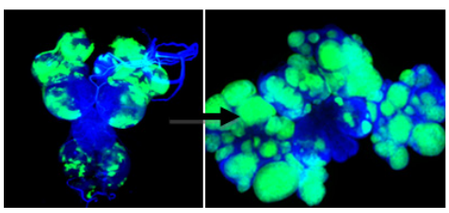
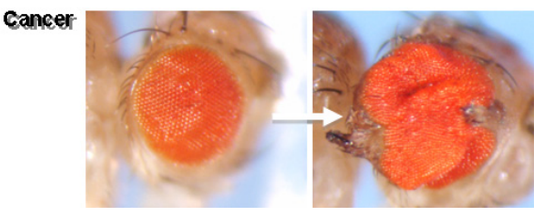
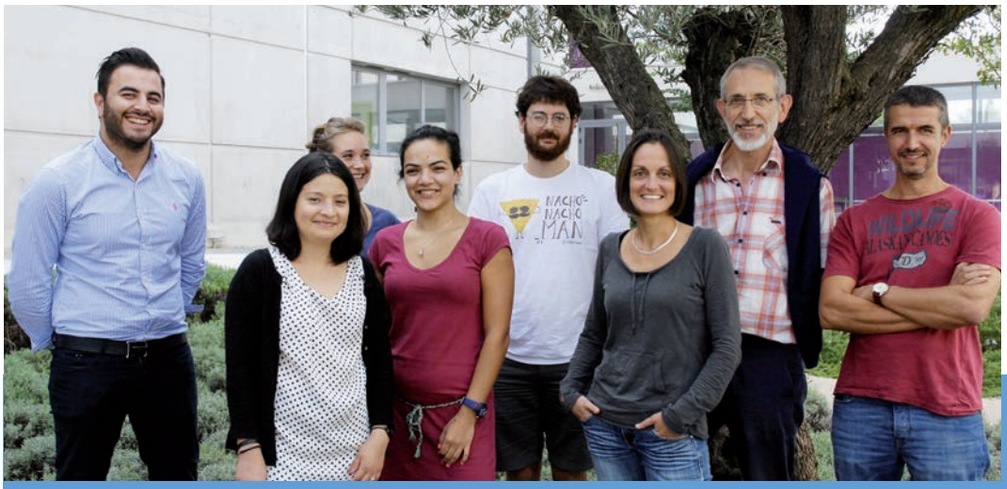


Figure 2. A graphical view presenting the idea of quantitative high throughput microscopy as a valuable tool for gene discovery. A stream of coloured cells is pouring into a "digital graph" decrypting complex high-throughput data in order to identify novel epigenetic components. In this data landscape, a little SUMO fighter is holding together a Polycomb body. In Gonzalez et al, we used a high-resolution microscopy genome-wide screen to identify proteins affecting the nuclear organization of Polycomb group proteins. Among the 129 identified candidate genes, we characterized SUMO components and showed that the level of PC SUMOylation regulates the 3D organization and the function of Polycomb components into nuclear bodies. Artwork by Julio Mateos-Langerak and Eneritz Agirre

Figure 3. Mutation of the polyhomeotic locus (second panel from the left) induces over-proliferation of the mutant tissue (in green, compare to control on the left). Most larvae die but around 10% survive and, in that case, the mutant tissue over-proliferates (the mutant eye in the second panel from the right is larger than wild type eye on the left) and forms tumors.







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RNA Silencing & Control of Transposition

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A large fraction of eukaryotic genomes is made of transposable elements (TEs). Their mobilization at high frequency is deleterious, resulting in high rates of mutations, chromosomal rearrangements, sterility and a variety of other disorders. Mechanisms that repress transposition and keep it at levels compatible with species survival have been selected during evolution. Small RNA pathways – referred to RNA interference (RNAi) pathways, play an essential role to limit TE proliferation. In the animal gonadal tissues, a specific class of small regulatory RNAs called PIWI interacting RNAs (piRNAs) are dedicated to TE repression. The piRNAs are bound by a subfamily of Argonaute proteins, the PIWI proteins to form the piRNA-interacting silencing complex (piRISC). Defects in the piRNA pathway lead to TE derepression, genomic instability and sterility. Recent studies draw an overview of the piRNA pathway. According to this, the genome accumulates transposon sequences in defined heterochromatic loci called piRNA clusters (Figure 1). These provide the RNA substrates for the piRNA primary biogenesis. An amplification cycle, called ping-pong or secondary biogenesis, involving the transcripts of both piRNA clusters and functional transposons boosts the piRNA production.

Our group was able to demonstrate that secondary piRNAs can be produced even in the absence of any functional TE mRNA owing to an amplification loop involving only defective heterochromatic TE sequences (Grentzinger et al., 2012). Surprisingly, unlike in the typical ping-pong model, such amplification loops do not seem to be initiated by primary piRNAs but by maternally transmitted secondary piRNAs. When deposited in the early embryo, this piRNA pool frequently initiates the amplification of piRNAs mediating, in the adult ovary, a strong repression of the I-element, a germline-specific TE. We have also demonstrated that the pool of maternally transmitted piRNAs affects the production of somatic Tirant piRNAs repressing Tirant in the ovarian somatic cells (Akkouche et al, 2013) (Figure 2). Our data clearly show that the maternally transmitted germinal piRNAs are the carriers of an epigenetic memory essential for both somatic and germline TE repression.

Using one of the most powerful genetic systems, the Drosophila model, our group focuses on piRNA biogenesis during the gonadal development, the role of maternally transmitted piRNAs over generations and the mechanism of action of the piRISC complex. For our studies we combine drosophila genetics, high throughput sequencing and proteomic approaches (Figure 3).

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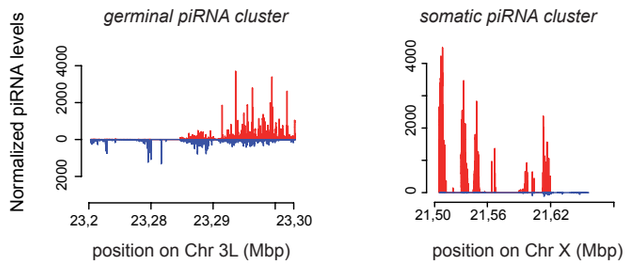


Figure 3 : General drawing of different approaches combined in our group.

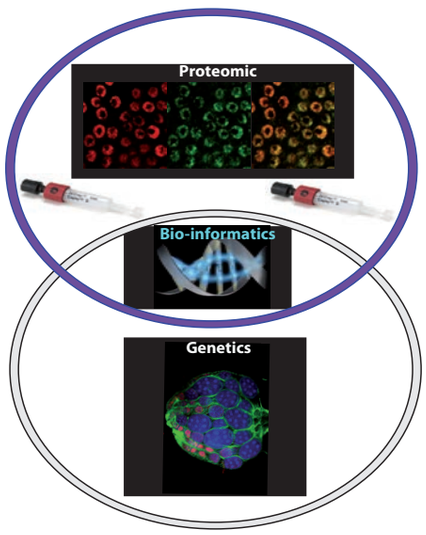


Figure 1 : Normalized profiles of ovarian piRNAs (sense: up (red); antisense: down (blue)) that map to a germinal and a somatic piRNA clusters. The y axis indicates the number of piRNAs the 5' end of which matches the x axis sequence at a given position

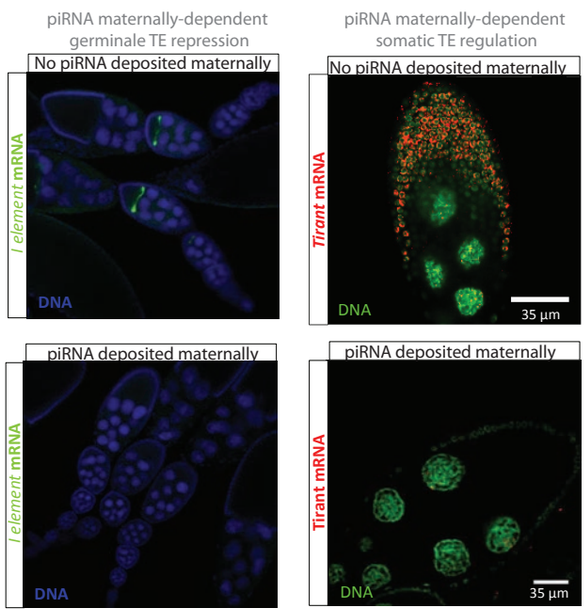
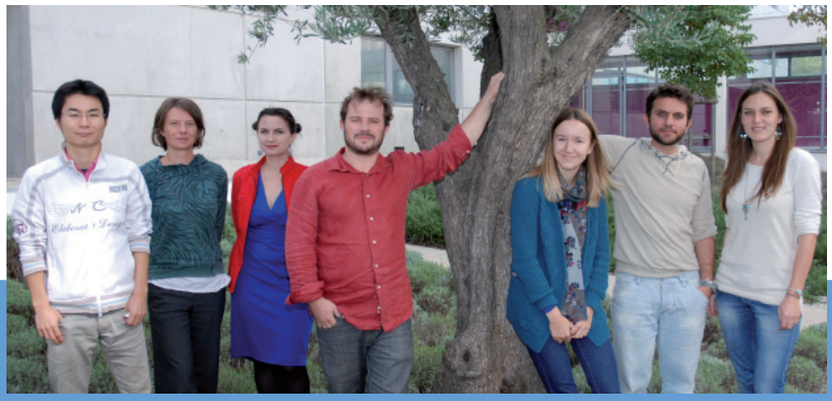


Figure 2 : RNA FISH on whole-mount preparations of ovaries with different amount of piRNA maternally deposited. On the left panels a FITC-conjugated antibody against a biotin-labeled antisense riboprobe specific for I-element sense transcripts. I-element sense transcripts were detected in green. Ovaries were counterstained with DAPI (blue). On the right panels an anti-dioxygenin-conjagated antibody against a dioxygenin-labeled antisense riboprobe specific for Tirant sense transcripts. Tirant sense transcripts were detected in red. Ovaries were counterstained with Sytox Green (green).



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Biology of Repetitive Sequences

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Chromatin can be viewed as a highly complex mixture of proteins and nucleic acids that orchestrate DNA-based processes in the eukaryotic genome. Most of the mammalian genome is assembled into heterochromatin, a 'closed' structure imposed by several enzymatic activities. Such activities act on histones and the DNA itself to impinge on transcription, replication or repair.

Most of the heterochromatic fraction of the genome can be found at critical loci. These include telomeres, repetitive sequences around centromeres and a portion (about half) of the gene units encoding ribosomal RNAs. Defects in the regulation of these loci have therefore disastrous consequences on cell identity and can lead to developmental problems, cancer, premature aging or immune deficiencies. How precisely heterochromatic enzymes affect the composition of target loci has remained elusive and research in our laboratory primarily focuses on this question.

To understand how heterochromatin acts at the molecular level, we are looking at the effect of abrogating important heterochromatic activities, such as histone and/or DNA methyl-transferases, on the overall composition of key heterochromatic loci (telomeres, pericentromeres and rDNA).

In particular, we are interested in:

(i) How telomere compositional changes upon loss of heterochromatin function can explain the appearance of the ALT (Alternative Lengthening of Telomeres) pathway observed in certain cancers.

(ii) How the situation at ALT telomeres can be compared to the changes observed at human satellite 2 sequences upon loss of DNA methylation in ICF cells. Indeed, satellite 2 regions recombine aberrantly and localize to PML bodies in ICF cells, a 'behavior' also observed in the case of ALT telomeres.

(iii) How pericentric heterochromatin is regulated by such enzymatic activities during development, differentiation and why such regulation matters for genome stability.

(iv) Characterizing the new SMCHD1 chromatin protein which possibly links DNA methylation and non-coding RNAs.

(v) How is rDNA expression regulated?

We have initiated these studies using a quantitative version of the PICh technology, qPICh, which couples SILAC with PICh. This approach allows the unbiased characterization of proteins bound to a specific locus in vivo (see figure). By correlating compositional and phenotypic changes at distinct loci, we hope our research will uncover important determinants of gene expression and genome stability. Importantly, because PICh has been adapted to quantitative approaches, we are now able to precisely monitor the dynamics of heterochromatin in vivo.

For more information, please, see:

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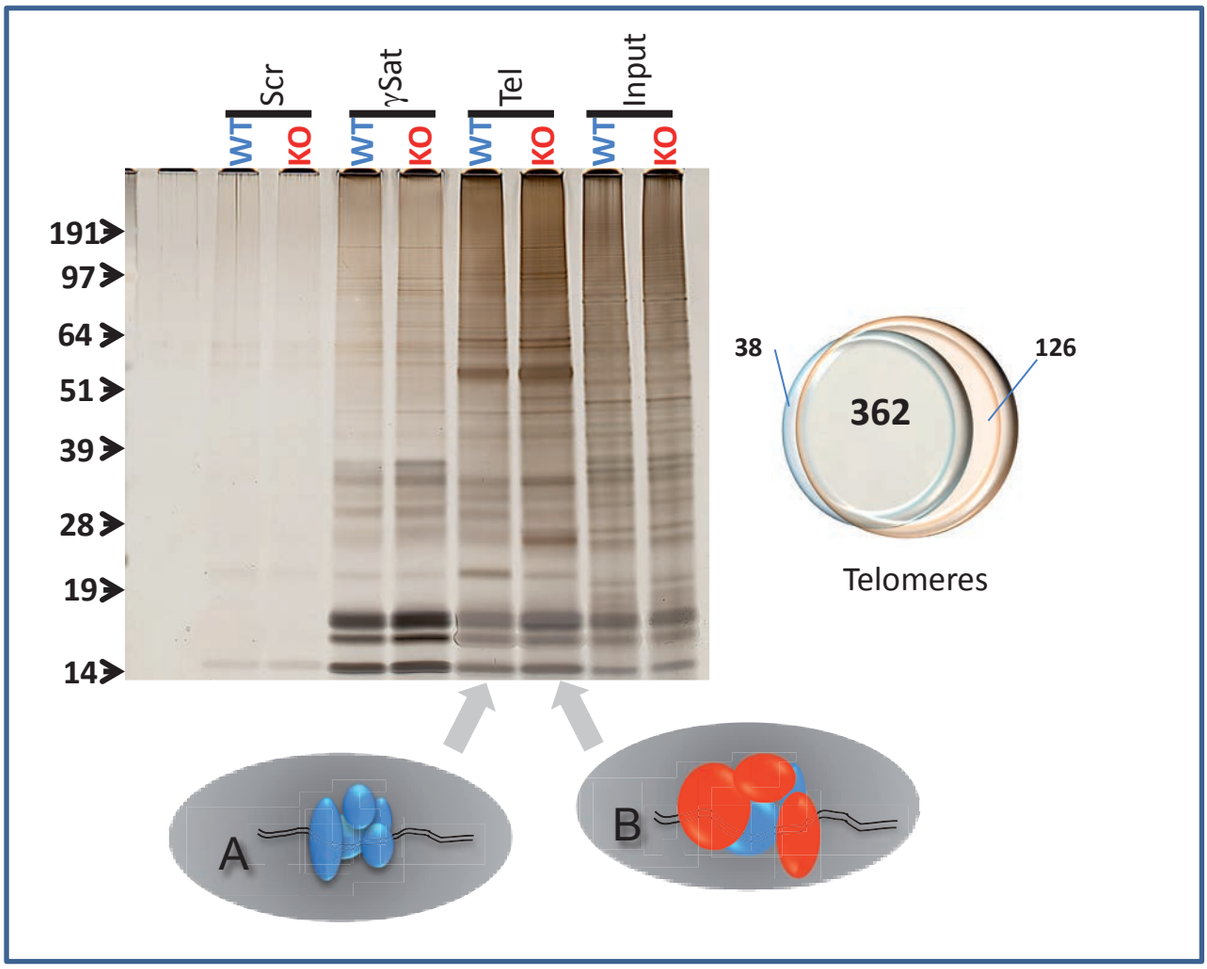
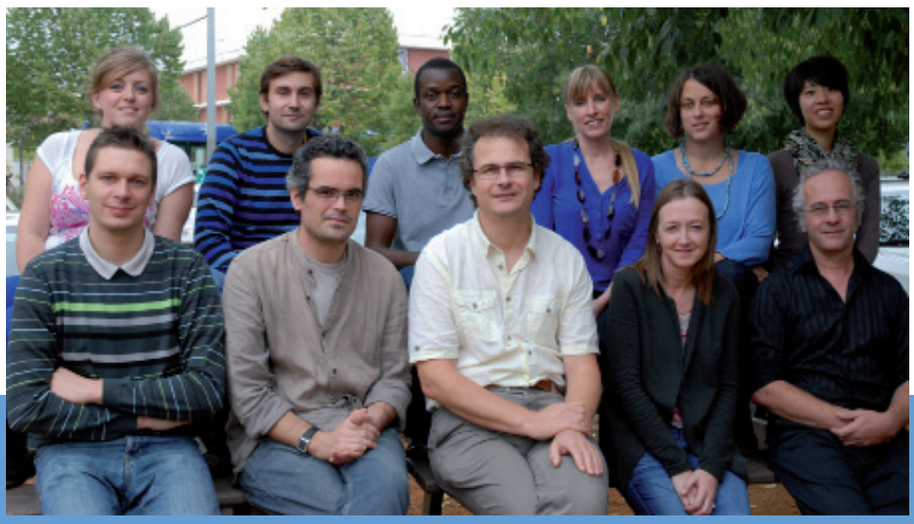


Purification of major satellites (γ Sat) and telomeres from mouse embryonic stem cells in WT or in Suv39h1+h2 K.O backgrounds. Composition of both loci is established in the two backgrounds, allowing to determine:

-the signature of constitutive heterochromatin in mammals, i.e. proteins found enriched in common at both loci (e.g. HP1 isoforms, etc…)

-the role of Suv39h in the biology of these targets: specific proteins are lost or gained at telomeres or pericentric chromatin in the absence of this important heterochromatin enzyme.



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Meiosis and Recombination

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In sexually reproducing species, meiosis allows the formation of haploid gametes from diploid cells. The halving of the DNA content results from a specialized cell cycle, where a single phase of DNA replication is followed by two divisions. In most species, the proper segregation of chromosomes at the first meiotic division requires connections between homologous chromosomes that result from reciprocal homologous recombination events or crossovers. Crossovers also generate new allele combinations and thus increase genetic diversity. The absence of crossover leads to segregation defects and sterility, and alteration of the meiotic recombination pathway can lead to genome rearrangements and aneuploidy.

Our group is investigating several aspects of the mechanism and regulation of meiotic recombination using the mouse as a model system. Meiotic recombination events are initiated by the formation of DNA double-strand breaks (DSBs), the repair of which leads to both crossovers and non-crossovers (gene conversion without crossover) (Fig. 1). Several hundred DSBs, catalyzed by the SPO11 protein, are formed at the beginning of the first meiotic prophase in mouse meiotic cells. SPO11 is homologous to the catalytic subunit of the Topo VI family of type II DNA topoisomerases, and is conserved among eukaryotes.

We are interested in understanding how the frequency and distribution of these DSBs are regulated, and how DSB formation and repair are coordinated.

We have recently discovered a major component that determines the sites where DSBs are formed in mammals: the *Prdm9* gene. This gene encodes a protein with a methyl-transferase activity and a tandem array of C2H2 zinc fingers. PRDM9 recognizes specific DNA motifs in the genome and is thought to promote trimethylation of lysine 4 of Histone H3 at these sites (Fig. 2). How does this protein actually function in vivo and how its activity allows the recruitment of the recombination machinery remains to be determined. In addition, a remarkable property of PRDM9 is its rapid evolution and diversity. We are currently investigating both its molecular and evolutionary features.

Meiotic DSB repair takes place in the context of chromosome axis which is thought to ensure a proper regulation for crossover formation. Interestingly, several proteins needed for DSB formation are located on chromosome axis. We have recently identified two such proteins in mice which are the orthologs of the yeast Rec114 and Mei4 proteins (Fig. 3). We are currently investigating the activities and functions of these proteins, and the role of this specific chromosome organization for DSB formation.

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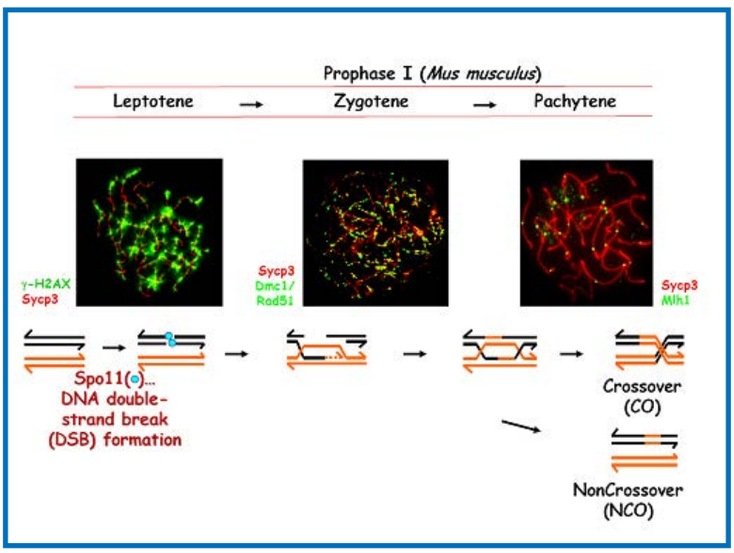
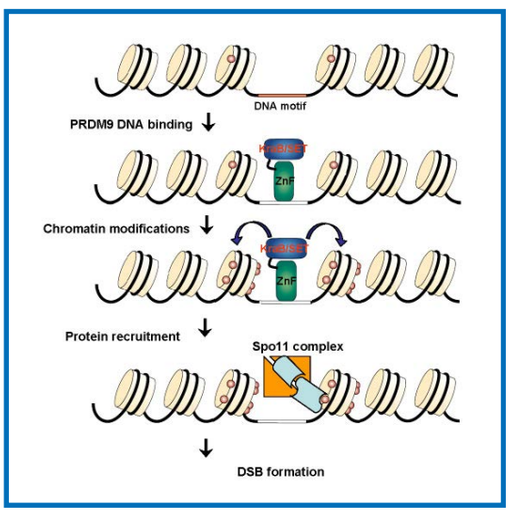
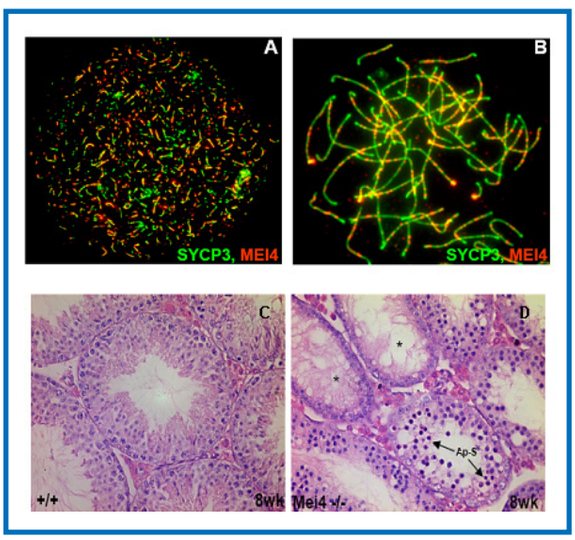


- **Fig.1**. DNA and cytological events during meiotic prophase.
Meiotic recombination is initiated by DSBs, which are catalyzed by SPO11 and visualized by the appearance of γH2AX (the phosphorylated form of H2AX). DSB repair, with the strand exchange activity of RAD51 and DMC1, leads to crossover (CO) and non- crossover (NCO) events. CO sites are visualized by the presence of MLH1 on chromosome axes (SYCP3) at the pachytene stage.



- **Fig.2**. Model of PRDM9 specification of meiotic recombination initiation sites in mammals.
PRDM9 binds to a DNA motif through its zinc finger domain and induces H3K4Me3 on adjacent nucleosomes (beige cylinder and histone post-translational modifications as red balls). Additional chromatin modifications and/or remodeling may take place and other proteins may be recruited. SPO11 is then recruited, binds to DNA and promotes DSB formation.



- Fig.3. *Mei4 is* essential for male and female fertility.
MEI4 (red) localizes as discrete foci along unsynapsed chromosome axes (labeled with SYCP3, green) at leptotene (A) and zygotene-like stages (B) in Spo11-/- and wild type (not shown) spermatocytes. Spermatogenesis in wild type (C) and Mei4 -/- (D) mice: meiotic arrest and apotosis are observed in Mei4 -/- mice. *, empty tubules; Ap-S, Apoptotic spermatocytes.

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Epigenetics and Splicing

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Alternative splicing is one of the most general and important biological processes in higher eukaryotic organisms. It affects more than 90% of human genes and it is essential for protein diversity. Each cell type is characterized by the subset of genes that are expressed and how they are spliced to an extent that any misregulation of the highly tissue-specific alternative splicing programs can lead to disease, such as cancer. Moreover, 15 to 20% of the mutations described to cause a disease are actually affecting splicing, highlighting the importance of understanding splicing regulation. However the mechanisms of cell-specific alternative splicing regulation are still largely unknown.

Unexpectedly, in the past 15 years, chromatin and epigenetic modifications have increasingly been shown to play an important role in the regulation of alternative splicing (Fig.1). In particular, we have shown that non-coding RNAs and histone marks can talk to the splicing machinery via recruitment of chromatin/splicing-adaptor complexes (Fig.2). We have found that alternatively spliced genes dependent on the ubiquitously expressed splicing factor PTB are enriched in a particular subset of histone marks depending on the cell-specific pattern of splicing. Modulation of these splicing-specific histone marks can change the pattern of splicing in a predictable way. The mechanism linking chromatin to the splicing machinery is enrichment of the chromatin-binding protein MRG15 along the alternatively spliced gene when enriched in H3K36me3. This chromatin-binding protein acts then as an adaptor and by protein-protein interaction induces recruitment of the splicing regulator PTB to the pre-mRNA, modulating in this way alternative splicing outcome (Fig.3). Importantly, in the absence of the adaptor protein, the relative levels of histone modifications along the alternatively spliced gene don't affect splicing anymore, proving the importance of this chromatin/splicing-adaptor system in regulating cell-specific splicing. Extending those studies, we have now found that splicing-specific histone marks cross-talk to each other, suggesting that epigenetic modifications regulate splicing in a combinatorial way. Finally, we have identified a long non-coding RNA, expressed in the antisense direction and within the alternatively spliced human gene FGFR2, responsible for the establishment of the splicing-specific chromatin signature that favors an epithelial-specific alternative splicing pattern, suggesting that non-coding RNAs are novel regulators of alternative splicing.

Currently, our group aims at the better understanding of the role of epigenetics and long non-coding RNAs in the onset and maintenance of tissue-specific alternative splicing programs. For that purpose we will use as an inducible cell reprogramming model system the epithelial-to-mesenchymal transition (EMT), involved in early development and cancer progression and metastasis. Combining classical and state-of-the-arte genome-wide -omics approaches we will depict the molecular mechanisms of regulation of tissue-specific alternative splicing programs with the final goal of modulating back to normal disease-related splicing patterns.

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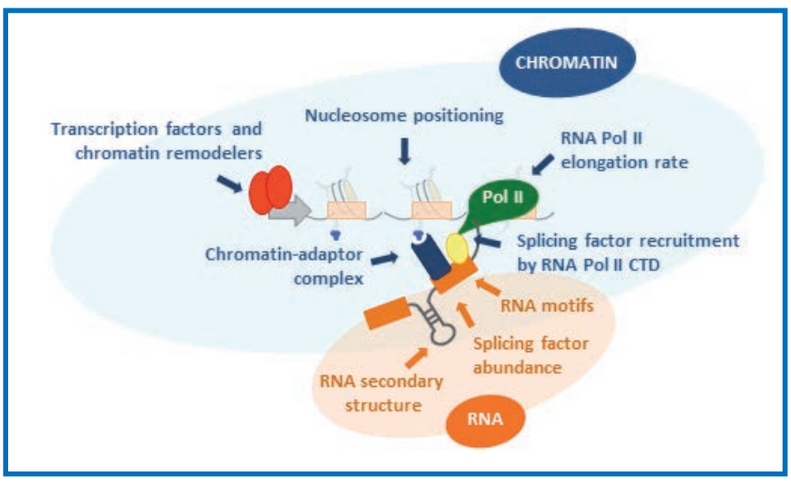

Figure 1.

An integrated model for the regulation of alternative splicing. Alternative splicing patterns are determined by a combination of parameters including cis-acting RNA regulatory elements and RNA secondary structures (highlighted in orange) together with transcriptional and chromatin properties (highlighted in blue) that modulate the recruitment of splicing factors to the pre-mRNA, all in an integrated way.

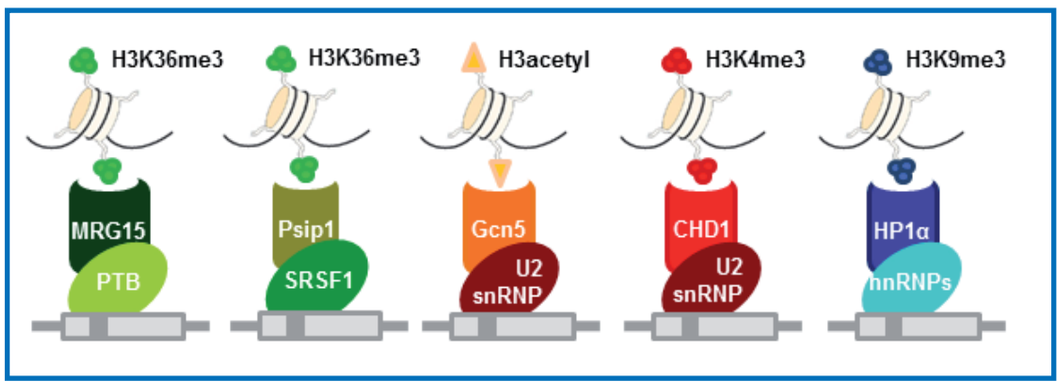

Figure 2. Chromatin/splicing-adaptor complexes. Chromatin talks to the splicing machinery via chromatin-binding proteins that by protein-protein interaction modulate recruitment of the splicing regulator to the pre-mRNA.

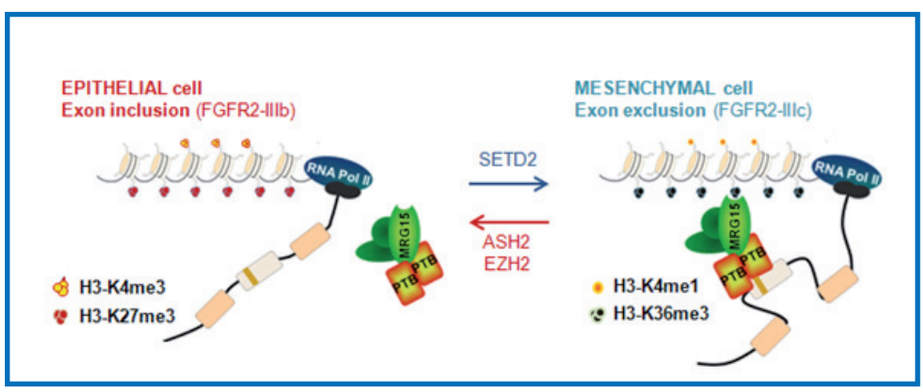

Figure 3. A chromatin-adaptor system for regulation of alternative splicing. Binding of the chromatin-adaptor MRG15 to an H3K36me3-rich chromatin favors recruitment of PTB to its target exon and the subsequent exclusion. H3K27me3 enrichment prevents MRG15 and thus PTB recruitment, favoring exon inclusion. Modulation of these histone mark levels by misexpressing key histone methyltransferases (SETD2, ASH2, EZH2) changes splicing outcome in a predictable way. However, in the absence of the chromatin-binding protein MRG15 histone marks cannot modulate splicing anymore, proving the importance of this chromatin-adaptor system in the regulation of splicing.

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Gene Regulation

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All organisms must regulate gene expression to achieve the silencing of certain genes and the activation of others during development and homeostasis. Deregulation of gene expression frequently has dire consequences, and can lead to pathologies such as cancer. The regulation of gene expression occurs at different levels, all of which depend on a multitude of factors. Chromatin is a primary regulator of gene expression.

Physical compaction of the genome into chromatin controls accessibility to the transcription machinery. Studies performed over recent years have revealed the enormous complexity involved in modifying chromatin to regulate gene expression. Once the genome becomes accessible, the engagement of the transcription machinery is a highly orchestrated process involving the recruitment of hundreds of factors that co-operate to achieve gene expression. Finally, transcription of a gene is linked to cellular processes required for the maturation and export of the mRNA in order to achieve gene expression.

The Gene Regulation Laboratory is interested in understanding the mechanisms that contribute to the silencing or activation of mammalian genes. We use the promoter of the human immunodeficiency virus (HIV-1) as a model to study gene regulation in mammalian cells.

Using this model, we have shown that the ubiquitin-proteasome system (UPS) strongly regulates HIV-1 transcription through recruitment of the 19S subunit to HIV-1 chromatin. We determined that a proteasome-associated protein, PAAF1, is a potent co-activator of transcription from the HIV-1 promoter. Ongoing studies are aimed at further characterizing the role of 19S and PAAF1 in transcription from HIV-1 and cellular promoters.

We have also recently shown that HIV-1 transcription is controlled by premature termination induced by the co-operative activities of microprocessor, Setx, Xrn2 and Rrp6. A subset of cellular genes and an endogenous retrovirus are also regulated by this pathway.

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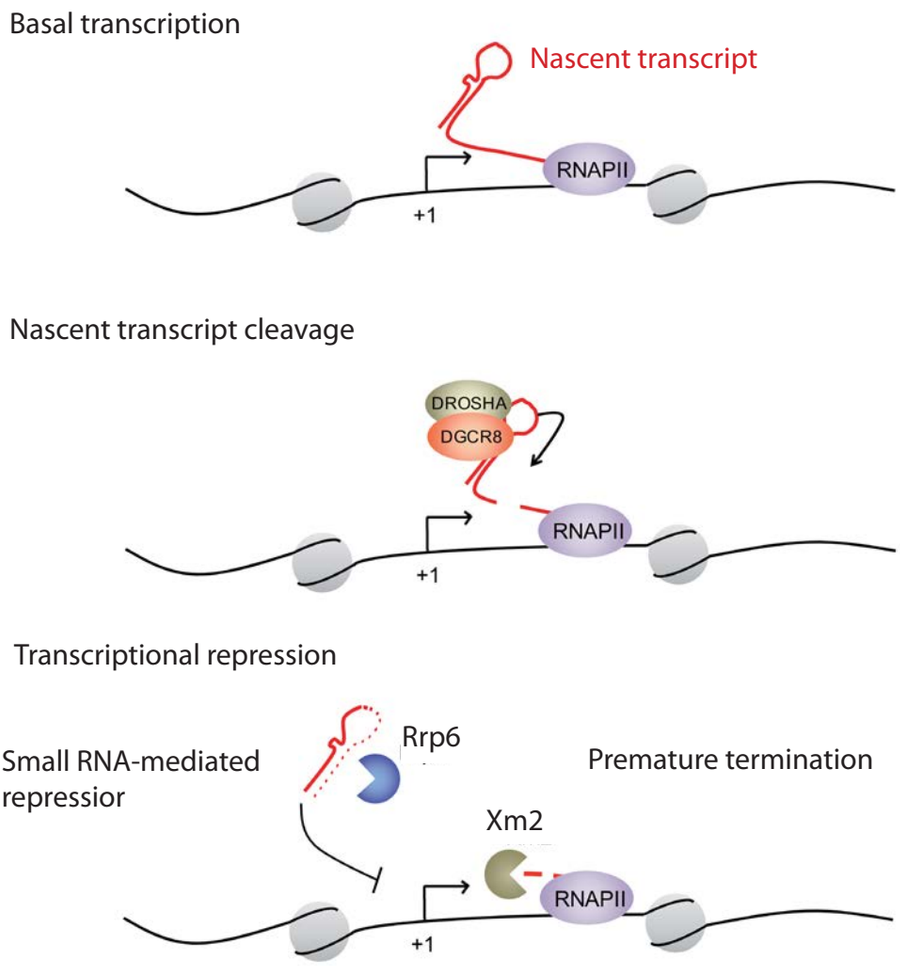

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Replication & Genome Dynamics

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Paradoxically, a major cell function such as the faithful duplication of the genome remains poorly understood in metazoans. During embryonic development chromosomes should be duplicated while maintaining memory of the specific ongoing transcription programs, because in multicellular organisms cell proliferation must not only deal with cell growth, but also with cell differentiation. In mammals, DNA replication starts at around 30 000-50 000 sites along chromosomes, known as DNA replication origins. As they do not share any detectable consensus sequence, unveiling their common features has remained a difficult challenge. In the last few years, we have been able to decipher most of the genetic and epigenetic signatures of mouse replication origins, and we now wish to determine their involvement in cell identity. We also aim at dissecting the molecular mechanisms used to build a chromosomal DNA replication origin, and wish to analyze how epigenetic mechanisms control the organization of chromatin domains for replication.

We have mainly used genome-wide approaches to identify replication origins (Figure 1) in mouse pluripotent stem cells and differentiating cells, as well as in Drosophila cells, and whole C. elegans. We have characterized several new features of replication origins, and found that they are conserved, including a new genetic element that we call the Origin G-rich Repeated Element (OGRE), which can form G-quadruplexes. These elements can form ectopic replication origins. We also analyzed the global organization of origins by DNA combing (Figure 1), and found that their usage was flexible. We suggested a flexible replicon model in which origins are organized in groups of adjacent potential origins that define a replicon. A single origin is activated in each replicon and the chosen one can vary from cell to cell. This flexibility in origin usage was dramatically enhanced when nuclei from differentiated cells were introduced into a mitotic embryonic context, in a reaction that mimics cells reprogramming (Figure 2).

In the second axis of our project, we exploit in vitro systems derived from Xenopus eggs (Figure 3) as well as mammalian cells to analyze the regulation of the initiation complex and its links with the cell cycle. During the past decade, our laboratory has characterized several replication factors, including Cdt1, MCM8, MCM9 and MCM-BP. We found that Cdt1 and geminin form a complex that acts as an ON/OFF switch at replication origins. We also reported two new members of the MCM helicase family, MCM8 and MCM9, and found that they play distinct roles during DNA replication, as well as forming a new complex involved in the control of recombination, DNA repair and animal fertility. We also identified a new Cdt1 domain required to prevent premature initiation of DNA synthesis in G2 phase. Different screens have been developed in order to identify and characterize new proteins regulating the replication initiation complex. Further information is available at: http://www.igh.cnrs.fr/equip/mechali/

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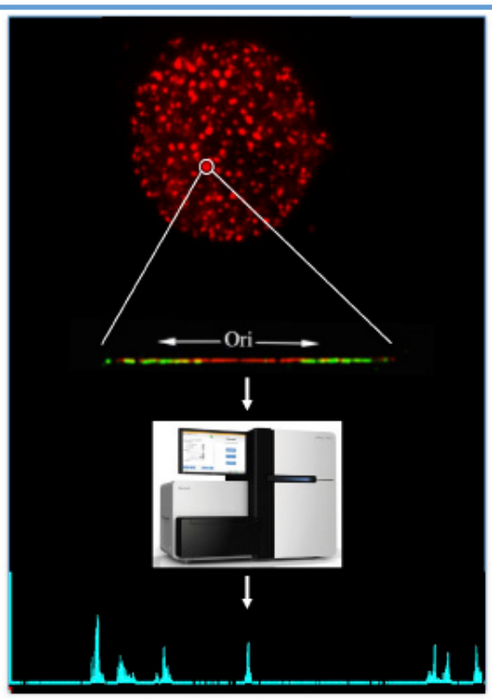


Fig.1. From replication foci to the replication origins code

A) nucleus, in which replication foci are labeled with BrdUTP followed by fluorescence imaging. B) When two consecutive pulses of labeling (red then green) are performed and the DNA combed on silanized glass, replication origins can be visualized, with the red labeling the origin and the green highlighting the progressing replication forks. C) Nascent strand isolation and high throughput DNA sequencing allow genome-wide identification of replication origin sequences, the positions of which (D) in the chromosomes can then be visualized.

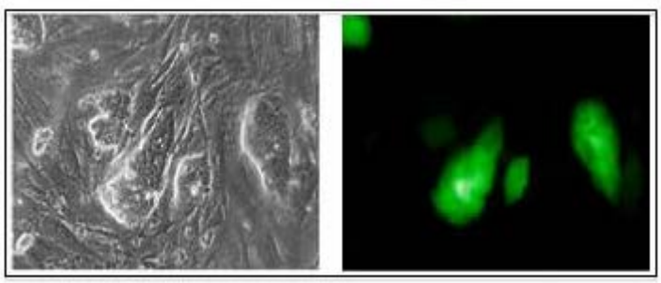


Fig.2. Mouse embryonic fibroblasts reprogrammed by Xenopus egg extracts express OCT4, a marker of pluripotency. Left, phase-contrast image. Right, fluorescence image showing cell clones expressing GFP under the control of the Oct4 promoter.

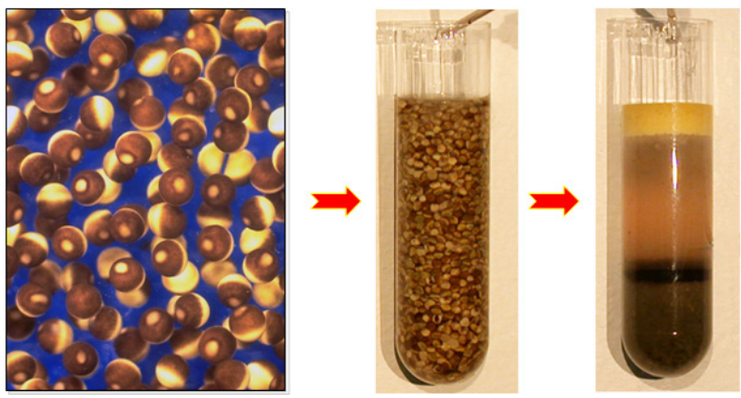
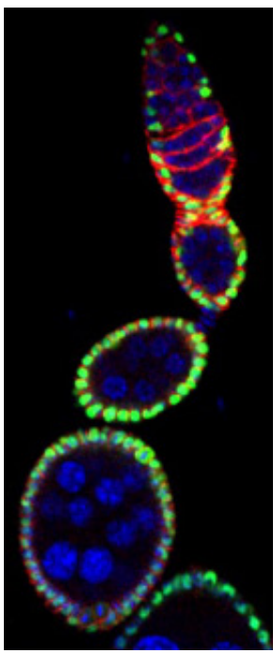


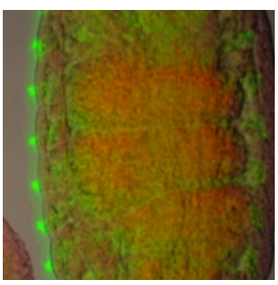
Fig.3. *From Xenopus eggs to DNA replication extracts*

Department of Genetics and Development

Director : Martine Simonelig



Martine Simonelig



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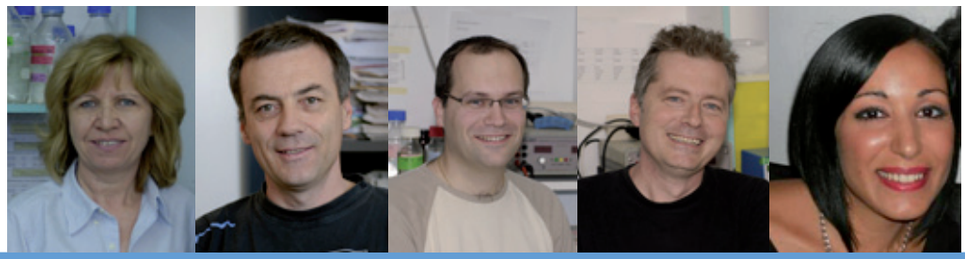
Developmental Genetics aims at understanding how the genetic information is translated into the production of many different cell types that are coherently organized in a complete organism. Groups in the Department of Genetics and Development are interested in various aspects of developmental genetics, from the establishment of cell polarity in the egg, to muscle differentiation, or the formation of an extremely complex structure such as the adult brain. Research topics in the Department include the identification of the molecular and signaling pathways that control the cell cycle as well as those involved in stem cell biology, in the development of the gonads and of the germ line and in muscle differentiation. Another line of research concerns the ligand/receptor interactions in axonal guidance during the development and function of the central nervous system. Several groups are interested in deciphering specific molecular mechanisms controlling developmental processes, such as the transcriptional code required for the dfferentiation of Sertoli cells, translational regulation and localization of maternal mRNAs in the oocyte and early embryo, RNA silencing by small non-coding RNAs (microRNAs and piRNAs), and post-translational modifications of tubulin. These fundamental biological questions are addressed using model organisms, including Drosophila and the mouse, and a variety of approaches. Groups in the Department have strong expertise in classical and cutting-edge genetic techniques, biochemistry, molecular and cell biology, advanced light microscopy and bioinformatics.

All the groups in the Department of **Genetics and Development** work towards understanding the molecular mechanisms of human diseases. Tumorigenesis is an important question addressed in the Department, through the utilization of cell and mouse models. Several groups have also developed Drosophila models of human diseases (e.g. muscular dystrophy, motoneural dysfunction, sterility), in which sophisticated genetic approaches can be applied to gain insights into the molecular pathways underlying these diseases. The analysis of multipotent stem cells showing regenerative potential is another important topic of research in the Department.

The Department of **Genetics and Development** has strong transversal interactions with other groups at the IGH and groups located in the close-by Institute of Functional Genomics that are also interested in some aspects of embryonic and germ line development, neurogenesis, muscle differentiation and regenerative potential of stem cells. The Department organizes each year the IGH Seminar Series on Genetics and Development.



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Development and Pathology of the Gonad

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The correct development of the reproductive organs, testis and ovary, requires the highly coordinated and regulated determination/differentiation of embryonic gonads, and maturation of the reproductive organs. Any abnormality in these processes during early embryo development, due to intrinsic genetic factors but also to environmental factors, will result in diseases. In male, testicular dysgenesis syndromes (TDS) lead to sexual differentiation disorders (gonadal dysgenesis (including sex-reversal), undescended testis (cryptorchidism), hypospadias), reduced sperm quantity and quality, semen abnormity (male infertility) and testicular cancer. In female, the gynecological implications of ovarian dysfunctions include cycle disturbances, anovulation, cyst formation and untreatable infertility and can derivate towards ovarian cancer.

In mammals, testicular differentiation is controlled by the Y-chromosome located gene Sry. This gene which encodes an HMG (High Mobility Group) domain-containing transcription factor of the SOX family, induces a variety of morphogenetic events, including cell proliferation, cell migration, Sertoli cell determination. At the molecular level, SRY directly activates Sox9 gene expression; SOX9 acts as the effector gene for Sertoli cell differentiation which itself induces the differentiation of the other gonadal cell lineages and subsequent testis cord formation. Our current research focuses on the cellular and molecular mechanisms involved in the formation of the embryonic gonad, particularly on the implication of the prostaglandin D2 (PGD2) signaling pathway in these processes and in the regulation of the expression and function of SOX9.

In the mouse embryonic testis, by ChiP-seq experiments, we identified the target genes for SOX9. We demonstrated the maintenance of SOX9 expression through the L/H-Pgds/PGD2 pathway that forms an autoregulatory loop with Sox9; this regulatory loop is independent of the FGF9/SOX9 loop but both contribute to maintain Sox9 expression and induce testis formation. On the other hand, we recently identified the implication of the PGD2 pathway in the induction of the mitotic arrest process in primordial germ cells and in the downregulation of pluripotency, contributing to the normal differentiation of the germ cell lineage.

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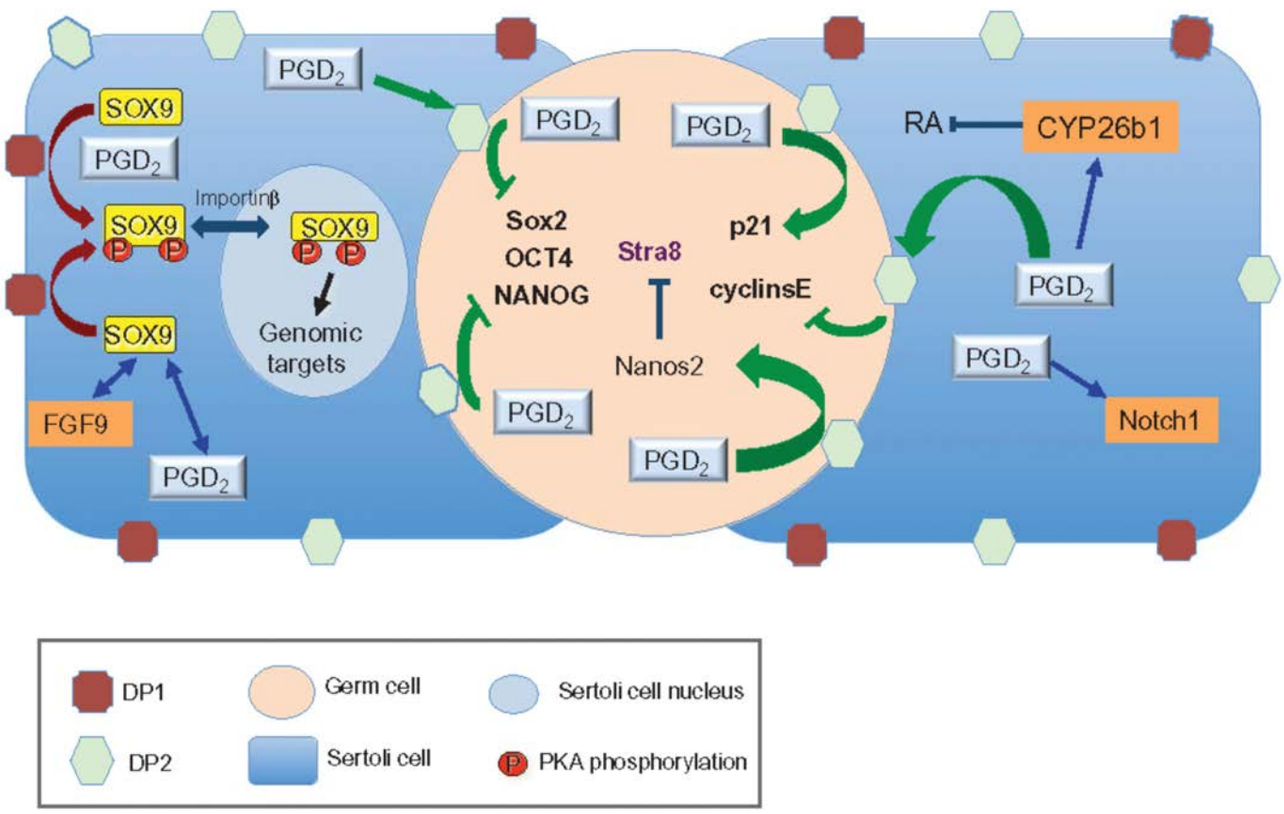
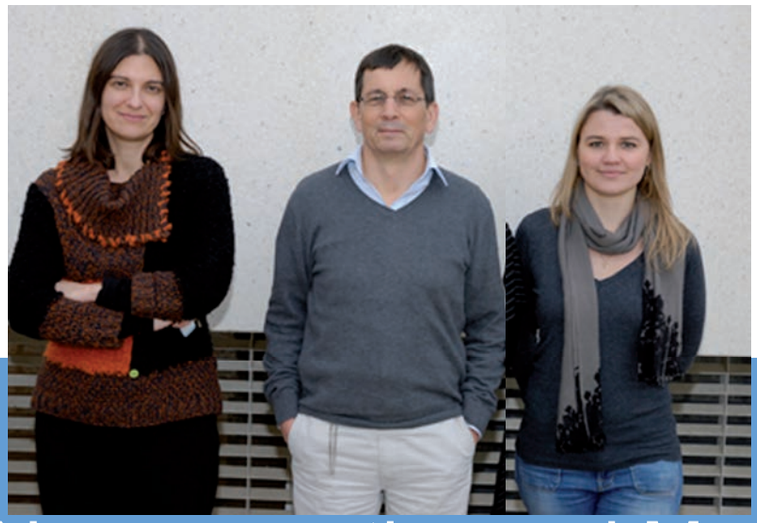


Roles of the prostaglandin D2 (PGD2) pathway in the somatic and germ cells differentiation in the embryonic testis.



INSTITUTE OF HUMAN GENETICS

Neurogenetics and Memory

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Developmental molecular genetics of Drosophila adult brain.

Developmental molecular genetics of Drosophila adult brain is an emerging science that is gaining momentum. We can reasonably foresee that the gene cascades at work during Drosophila brain development are conserved in mammals as well. In Drosophila, novel techniques are now available and allow working efficiently on this topic. Mushroom bodies (MB) may be considered as the analog of the mammalian hippocampus and are an excellent model for studying brain development. Each of the 4 MB neuroblasts generates, in a sequential fashion, three distinct classes of neurons. First the γ then the α'β' and finally the αβ neurons appear during development. MBs are essential for several forms of learning and memory. We have introduced in the laboratory a memory paradigm based on male courtship behavior. Therefore we are able to correlate the developing brain structure with its function.

I) Genetic control of neuronal remodeling during brain and neuro-muscular junction (NMJ) development.

Neuronal remodeling occurs widely during the construction of both invertebrate and vertebrate nervous systems. Alteration of neuronal remodeling is also a key aspect of neurodegenerative diseases, such Alzheimer's. MB γ neurons arise during early larval stage and undergo pruning at metamorphosis. We have shown that ectopic expression of the HR39 nuclear hormone receptor blocks γ axon pruning and impairs short-term, but not long term, memory. Pruning is also present at NMJ during metamorphosis. This mechanism is still poorly understood and hardly studied. We have described in detail this pruning and showed that some of the molecular actors are conserved between these two pruning systems.

II) Genetic control of axonal growth and guidance during brain development.

Neurons often innervate multiple distinct targets via axon branching. However, how differential guidance of branched axons occurs remains largely unknown. One MB neuron typically sends an axon, which at a precise location of its trajectory splits in two processes (branched axon). Thus, the MB provides a relatively simple single branched model in which to understand the mechanisms of differential branch guidance. We have identified four relevant genes for this branch axonal guidance: the derailed (drl) receptor type tyrosine kinase (orthologue of the oncogene H-Ryk), its ligand Wnt5 (orthologue of the oncogene/tumor suppressor Wnt5a), Drl-2 one of the two drl paralogue and more recently Appl, homologue to human APP, involved in Alzheimer's disease. The axons integrate molecular information provided by the ligand and the three receptors (intrinsic and extrinsic) for their guidance.

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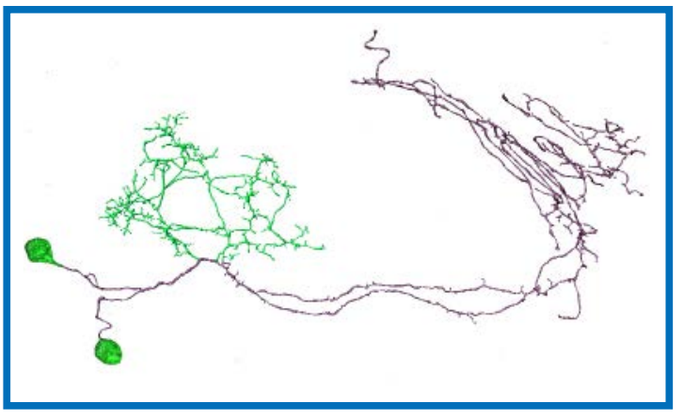


Fig 1 : 2 γ neuron clone in a larval brain (in green the cell bodies and the dendrites).

Fig 2 : Larval brain with DRL receptor in red and FASII in green (after the cover of September 2007 issue of Development)

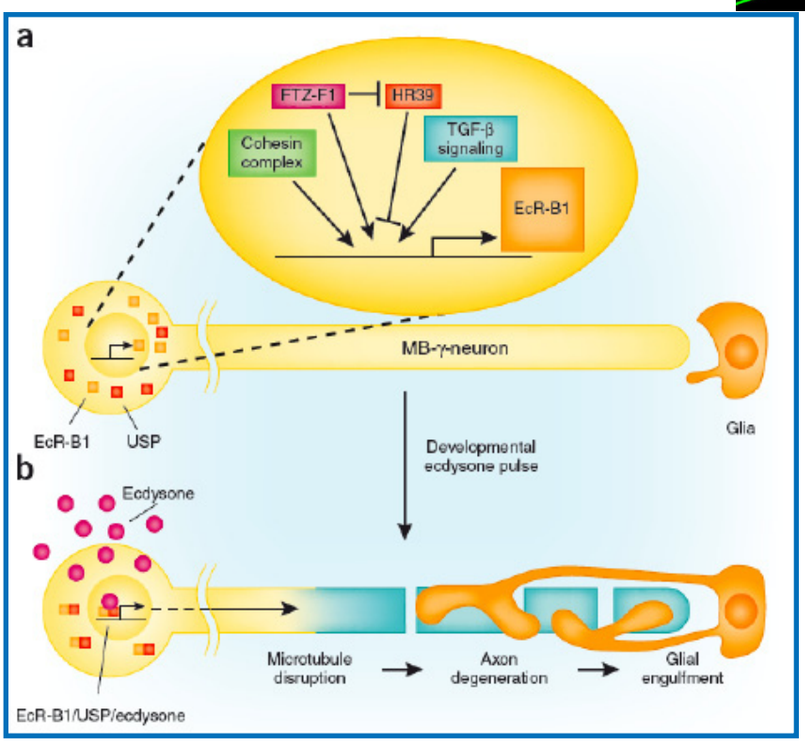


Fig 4 : Model for EcR-B1 activation in MB neuron remodeling. After a News and Views by Awasaki and Lee introducing Boulanger *et al*., 2011.

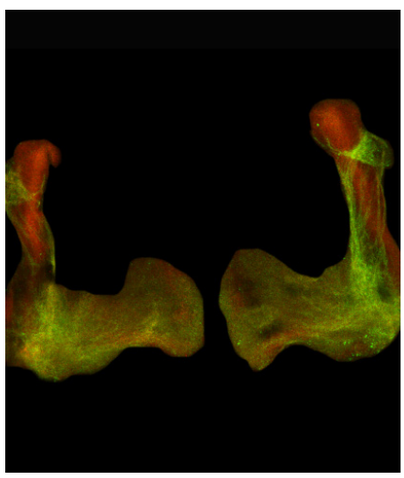
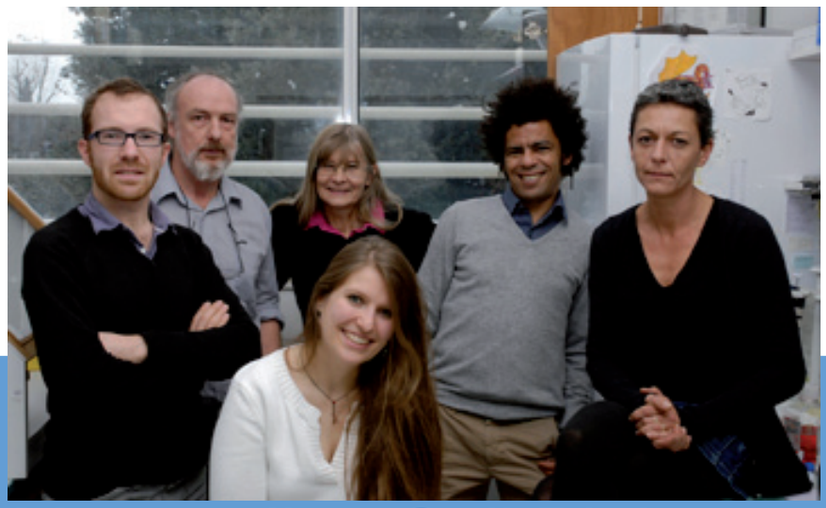


Fig 3 : Adult MB with un-remodelled γ axons (green) and normal αβ axons (red).



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Mammalian Cell Biology

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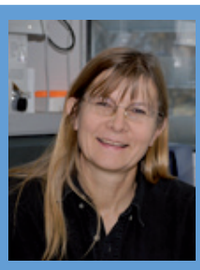

Anne Fernandez
Ned Lamb
Research Directors CNRS

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Céline Franckhauser,
Engineer CNRS

PhD students
Romain Davaze,
Violeta Mitutsova

Our research themes are focused on the control of mammalian cell cycle and differentiation using cell biology approaches on human and rodent cultured cell lines and primary adult stem cells isolated from skeletal muscle. We examine protein post-translational modifications, in particular phosphorylation, as essential components in the transduction of signals associated with cell exit from quiescence, activation into proliferation and irreversible arrest into terminal differentiation.

In the process of myogenic differentiation we have examined the role of the insulin/IGF pathway and the downstream activator PKB/Akt protein kinase family. Our studies are focusing on differentiating potential interacting partners, such as p21 and CTMP, and the specific action of Akt1 and Akt2 isoforms in proliferating and differentiating myogenic cells. We had previously shown that p21 specifically bound Akt2 and recently mapped the p21 interaction domain on Akt2 to a 27 C-terminal amino acid sequence of Akt2. We are currently mapping the same interaction domain for CTMP that appears to preferentially interact with Akt1.

Our complementary major research theme involves the isolation and characterization of a population of skeletal muscle-derived stem cells, MDSC, capable of multipotent differentiation particularly into spontaneously beating cardiac muscle cells and neuronal lineages. In collaboration with IGF teams, we are analyzing the in vivo multi-lineage differentiation and physiological repair potential of MDSC using mouse models of targeted diseases and lineage-specific tracking of MDSC differentiation in particular towards cardiac and beta-pancreatic differentiation. Beating myocytes differentiated from MDSC in vitro were shown to be fully functional pacemaker cells such as those in the sino-atrial node (SAN) of the heart and transplantation experiments in mutant mice revealed that multipotent MDSC improved heart rhythm while engrafting into the SAN of severely bradycardic mice thus proving a very promising repair and regeneration potential. MDSC being, unlike iPSC or ESC, non-teratogenic, can be safely transplanted without need to manipulate or induce them into a pre-differentiated stage, thus preserving their high plasticity, survival and migratory potential. In this context, we are investigating the bio-distribution of MDSC after IP or IV injection into living mice with the objective of identifying if and how the transplanted cells are specifically recruited and home to damaged target tissues.

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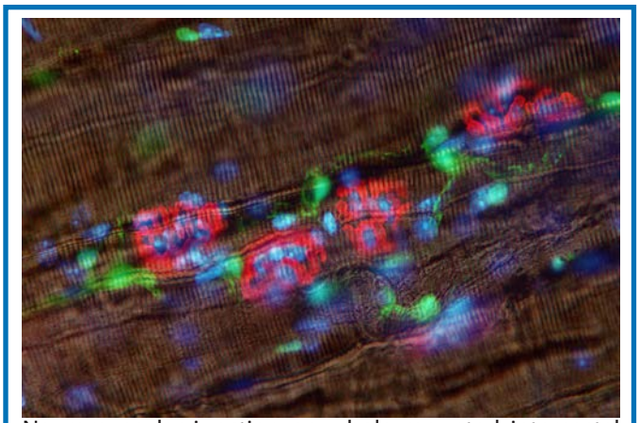
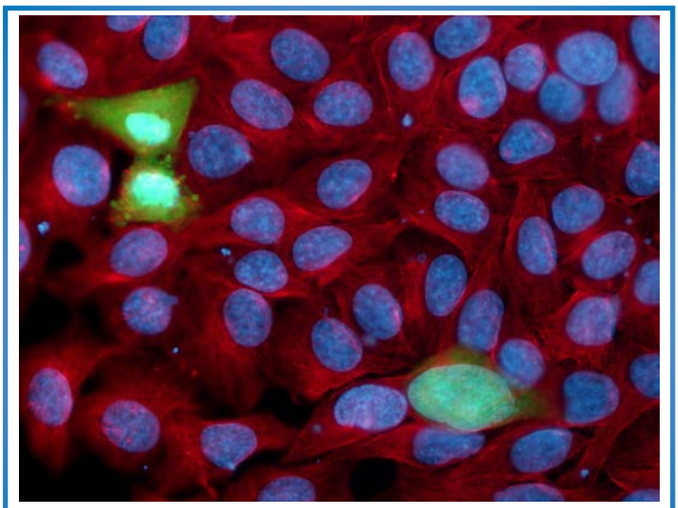
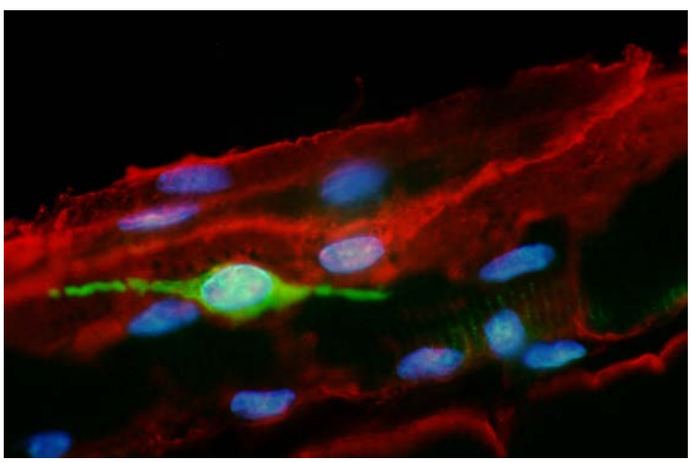


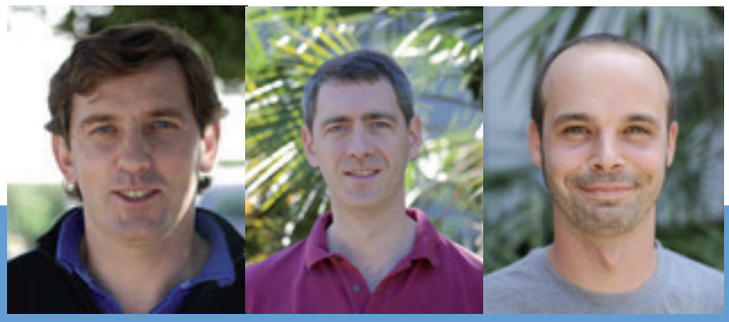
Neuromuscular junction on whole mounted intercostal mouse muscle with red bungarotoxin, eGFP-Nestin expressing cells, blue nuclei and phase contrast image of muscle fibers



eGFP-Akt expression in human fibroblasts with tubulin staining in red and nuclei (DNA) in blue



Rat muscle cryosection with Alexa-green Nestin , Alexa-red Laminin and blue DNA staining



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Tubulin Code

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Microtubules (MTs) are essential cytoskeletal elements composed of alpha- and beta-Tubulin heterodimers. They are involved in a range of cellular functions including cell division, maintenance of cell shape, intracellular transport as well as cell motility. The mechanisms that allow MTs to perform such a diverse range of functions are poorly understood, but it is clear that each specific MT function requires the recruitment of a particular set of MT-associated proteins (MAPs). Strikingly, many MAPs interact with the C-terminal tails of Tubulins, which are known to protrude from the MT surface and to undergo several unusual post-translational modifications (Westermann and Weber, 2003). Such Tubulin C-terminal modifications include the removal of the very C-terminal tyrosine from alpha-Tubulin and two so-called poly-modifications, namely poly-glutamylation and poly-glycylation, which consist in the addition of side chains of either glutamate or glycine residues to the C-terminal tails of both alpha- and beta-Tubulin. The combination of the different Tubulin C-terminal modifications together with the fact that the side chains generated by the poly-modifications vary in length provides a high potential for encoding patterns on the MT surface that might recruit specific MAPs and allow the functional adaptation of MTs. In addition, since all these modifications have been shown to be reversible, they permit rapid changes in the MT properties.

Given the range of signals that the Tubulin C-terminal modifications can generate, it is not surprising that particularly high levels of these post-translational marks are present in complex and sophisticated MT-based structures, such as the ones found in neurons or in cilia and flagella. However, until recently, very little was known about their functions, mainly due to the lack of knowledge about the modifying and demodifying enzymes involved. For a long time, the only known enzyme involved in Tubulin modifications was Tubulin Tyrosine Ligase (TTL) (Ersfeld et al., 1993), which re-attaches the C-terminal tyrosine to detyrosinated alpha-Tubulin. During the last few years, we have identified the enzymes involved in Tubulin poly-glutamylation and poly-glycylation and shown that they belong to the TTL-like (TTLL) protein family (Janke et al., 2005; Rogowski et al., 2009; van Dijk et al., 2007). Recently, we have also discovered several deglutamylases, the enzymes catalyzing the removal of poly-glutamylation, as members of the cytosolic carboxypeptidase (CCP) family (Rogowski et al., 2010).

The main goal of our research is to understand how the three Tubulin C-terminal tail modifications (detyrosination, poly-glutamylation and poly-glycylation) regulate MT functions. The only cell types where all these modifications coexist are ciliated and flagellated cells. Cilia and flagella are involved in a number of cellular processes that range from motility, development, fluid movement to signal transduction. Recently, cilia moved into the spotlight due to the growing number of diseases associated with their defects. Defective cilia lead to a wide variety of disorders, including hydrocephalus, primary ciliary diskinesia, polycystic kidney disease, situs inversus, retinal degeneration, obesity, hypergenitalism and polydactyly as well as cancer (Sharma et al., 2008).

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Quite often, cilia-related diseases occur in combination with male sterility, thus underlying the functional and structural similarities between cilia and flagella. Hence, we are using sperm development in Drosophila and mice as a model system to study the roles of Tubulin modifications in the assembly and functions of cilia and flagella.

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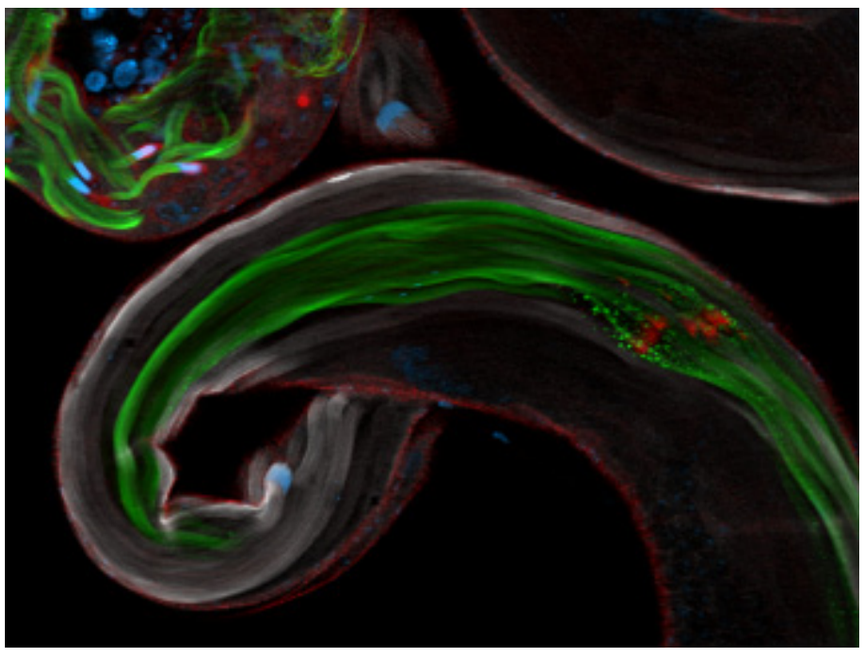

Immunofluorescence of wild type *Drosophila* testis. Actin is stained with TRIC-conjugated phalloidin (red) while polyglycylated tubulin is revealed with PolyG antibodies (green). The nuclei are stained with DAPI (blue) and detyrosinated tubulin is labeled by delta1-tubulin antibodies (grey).

INSTITUTE OF HUMAN GENETICS

Systemic impact of small regulatory RNAs

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MicroRNAs (miRNAs) are small regulatory RNAs that repress specific target genes through base-pairing with the target mRNA. Computational analyses aim at identifying miRNA targets by searching miRNA binding sites that have been conserved in evolution; such algorithms predict thousands of miRNA targets in animal models. While they seem to have many targets, miRNAs usually repress them very modestly (less than 2-fold in general), hence they have been proposed to fine-tune these numerous genes, precisely setting protein abundance to its optimal level.

We proposed an alternative hypothesis: as most genes in animals are robust to small changes in gene expression (for example, most genes are haplo-sufficient in animals), we expect most predicted "miRNA targets" to be insensitive to the miRNA-mediated <2-fold repression. Yet their interaction with miRNAs has been conserved in evolution, hence it must have a function: we proposed that these "pseudo-targets" rather act as competitive inhibitors, repressing miRNAs by titrating them. Just a small subset of predicted targets would actually be functionnally targeted by miRNAs: these "real targets" would be the most dose-sensitive genes among predicted targets (Seitz, 2009).

Our laboratory is confronting the two hypotheses, testing their predictions by experimental and computational methods (see figures 1 and 2). Our work suggests that the number of real miRNA targets (hence, the physiological impact of miRNAs) has been vastly over-estimated.

In addition to that major project, our group is also involved in several collaborations related to the biogenesis or the function of small regulatory RNAs: understanding the molecular mechanisms of miRNA biogenesis (collaboration with Y. Tomari, university of Tokyo; Kawamata et al., 2009; Tsutsumi et al., 2011); dissecting the origins of phenotypic robustness to perturbation of gene regulators (collaboration with J. Turner, MRC, London); exploring small regulatory RNA biology in emerging model organisms (collaborations with P.D. Zamore, UMass Medical School; U. Technau, university of Vienna; D. Tagu, INRA; and H. Escriva, CNRS and UPMC) (see figure 3).

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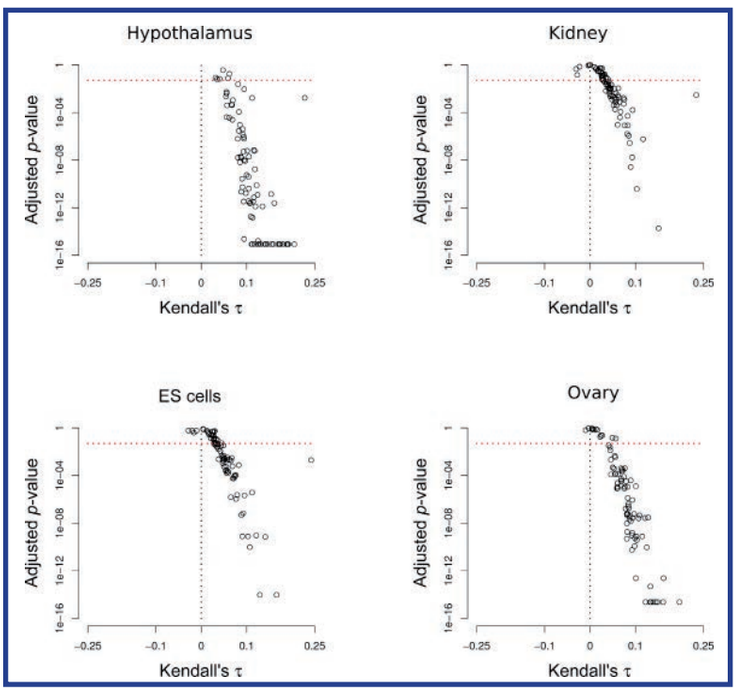

Figure 1. For predicted miRNA targets, mRNA abundance correlates positively with miRNA binding site conservation. Each point represents a murine miRNA family. Correlation between mRNA abundance and conservation of miRNA binding sites was assessed across all predicted miRNA targets (these volcano plots show the correlation coefficients and their p-values). mRNA abundance was extracted from published microarray datasets and miRNA binding site conservation was evaluated using TargetScan's «probability of conserved targeting» (described in Friedman et al. (2009) Genome Research 19: 92). A positive correlation between mRNA abundance and target site conservation was predicted by the pseudo-target hypothesis, and cannot be explained by the genome-wide fine-tuning hypothesis.

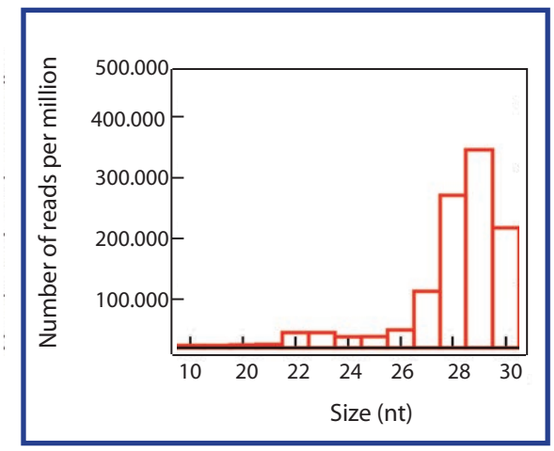

Figure 3. Size distribution of small RNAs from Nematostella vectensis early planula. Small RNAs were sequenced on an Illumina GAII sequencer. Genomic annotation reveals three classes of small RNAs: piRNAs (25 to 30 nt long), miRNAs (22 to 23 nt long) and endogenous siRNAs (20 nt long).

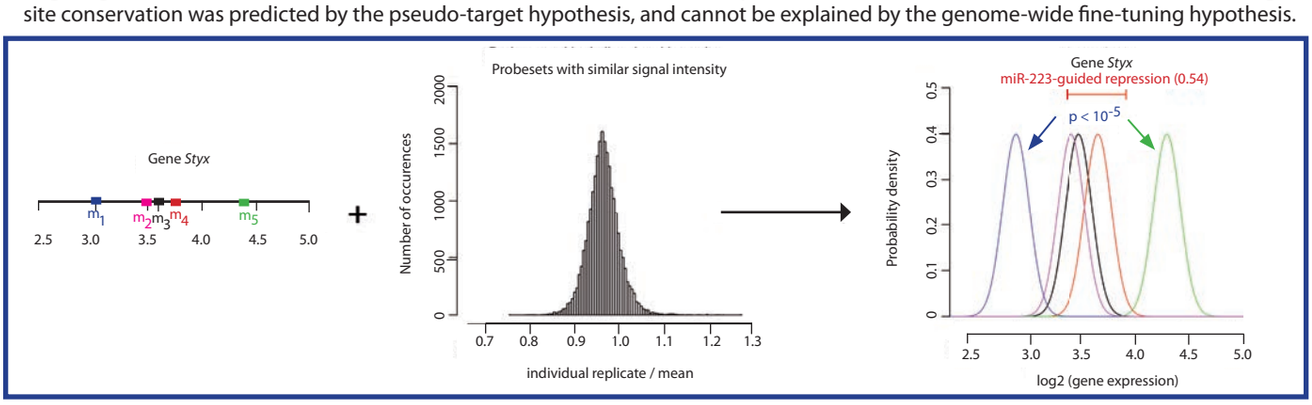

Figure 2. For most predicted miR-223 targets, inter-individual fluctuations in a wild-type population exceeds miR-223-guided repression. We measured gene expression in neutrophils in five wild-type mice by microarray (left panel) and measured technical variability of the complete experimental procedure (middle panel). The right panel shows probability distributions of the underlying biological values for the expression of that gene (calculated based on the measured technical variability of the experiment). The p-value (shown in blue) measures the probability that the underlying differences in gene expression among the five mice is smaller than miR-223-guided repression of that gene (shown in red; taken from Baek et al. (2008) Nature 455: 64). For 168 out of 189 analyzed miR-223 predicted targets, inter-individual variations in gene expression appear to be larger than miR-223-guided repression ($p < 0.05$), suggesting that these genes are not functionnally affected by miR-223.

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mRNA Regulation and Development

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Post-transcriptional regulation has a huge impact in the control of gene expression and is crucial for many developmental processes. We are using Drosophila, a genetically tractable organism, as a model to investigate the regulation of mRNA 3'-end processing and poly(A) tail length, and its role in the control of gene expression during development and disease.

Translational control of early development by poly(A) tail length: cytoplasmic polyadenylation and deadenylation

In many species, early steps of development occur in the absence of transcription and depend on maternal mRNAs and on their regulation at the level of localization, translation and stability. A major mechanism of translational control and mRNA stability involves changes in the length of mRNA poly(A) tails. Poly(A) tail elongation by cytoplasmic polyadenylation leads to translational activation, whereas poly(A) tail shortening by deadenylation leads to mRNA decay, or translational repression. In Drosophila, regulation of mRNA poly(A) tail lengths is crucial for anterior-posterior patterning of the embryo since this regulation controls the synthesis and localization of morphogens: Bicoid at the anterior pole and Nanos at the posterior pole. We are investigating the molecular mechanisms and the roles of this regulation during oogenesis, meiosis, stem cell biology in the female germline and axis formation in the embryo.

We are currently studying the role of the small non-coding RNA silencing pathways in the decay of maternal mRNAs in the early embryo. We have shown that the piRNA (Piwi-interacting RNA) pathway is involved. This pathway is known to repress the transposition of transposable elements. Moreover, piRNAs are themselves produced from transposable elements. Our finding proposes the first example of a role for transposable elements through piRNAs in gene regulation and embryo patterning.

Drosophila as a model for understanding human diseases: the Drosophila model of oculopharyngeal muscular dystrophy (OPMD)

Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset syndrome characterized by progressive degeneration of specific muscles. OPMD is caused by short GCG repeat expansions within the gene encoding the nuclear poly(A) binding protein 1 (PABPN1) that extend an N-terminal poly-alanine tract in the protein. PABPN1 has a role in mRNA polyadenylation. Mutant PABPN1 molecules aggregate as nuclear inclusions in OMPD patients' muscles. We have generated a Drosophila model of OPMD that recapitulates the features of the human disorder: progressive muscle degeneration and formation of PABPN1 nuclear inclusions. Strikingly, the RNA binding domain of PABPN1 and its function in RNA binding are required for muscle degeneration, demonstrating that OPMD results from an intrinsic property of PABPN1. We are using this model and a set of complementary genetic and molecular approaches to identify the molecular mechanisms underlying the disease. We are also investigating the potential of novel therapeutic strategies, including the utilization of anti-PABPN1 intrabodies, and the identification of beneficial drugs.

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Figure 1: *Drosophila* ovarioles showing the presence of germline stem cells (GSCs) at the anterior tip of the germarium in wild-type ovarioles (long structures) and the lack of GSCs in ovarioles mutant for the CCR4 deadenylase (short structures). Staining was with DAPI (blue), anti-Vasa as a marker of germ cells (red) and 1B1 to label the spherical spectrosome in GSCs (green). The CCR4 deadenlylase is required for GSC self-renewal through its role in translational repression of differentiation mRNAs.

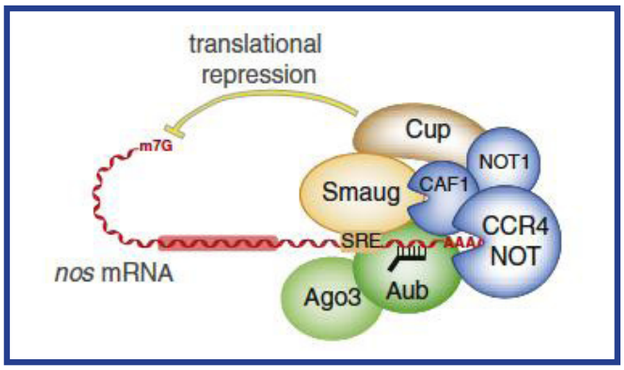


Figure 2: Model of *nanos* mRNA regulation by the piRNA pathway. SRE: Smaug recognition elements. The Smaug RNA binding protein (orange) binds to the SRE and recruits the deadenylation complex (blue). piRNAs (black comb) from retrotransposons target *nanos* 3'UTR and guide the interaction with Argonaute proteins (green) which stabilize the complex.

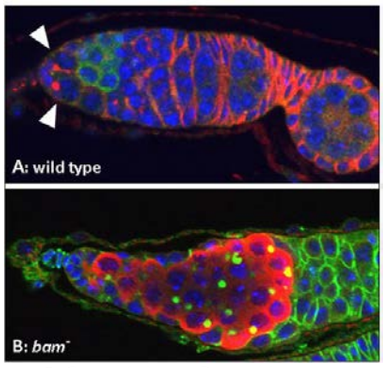
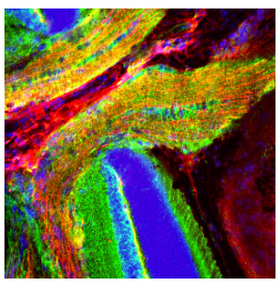


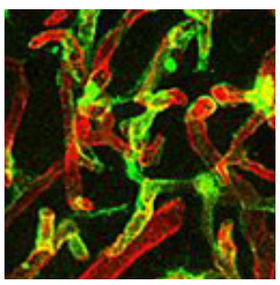
Figure 3: Germaria in the *Drosophila* ovary.
A: wild-type. All germline cells derive from two germline stem cells (marked by a dot with the 1B1 marker (red), arrowheads). Bam (green) is expressed in cystoblasts and is required for their differentiation. DAPI (blue).
B: In the *bam* mutant, germline stem cells cannot differentiate and form a tumor of stem cells. 1B1 (green). Vasa marks all germline cells (red). DAPI (blue).

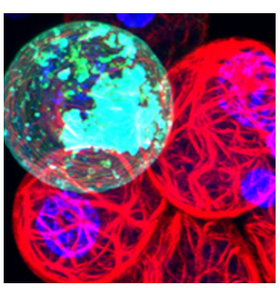
Figure 4 : *Drosophila* thoracic muscles.
A: diagram of dorso-longitudinal indirect flight muscles.
B: normal dorso-longitudinal muscles in a control fly.
C: *Drosophila* model of OPMD. Muscles expressing mutant PABPN1 degenerate (arrowhead).

Department of Molecular Bases of Human Diseases

Director : Monsef Benkirane







MRI Montpellier

Research in the department of Molecular Bases of Human Diseases strives to shed light on the etiology of cancer and AIDS. Supported by strong collaborations with the academic hospitals, our objective is to translate novel biological concepts and molecular insights into new therapies.

Genome instability and cancer.
Four research groups use complementary model systems (yeast, Xenopus and human cells) to explore two major topics in cancer biology: the origin of genomic instability in cancer development and the cellular responses to DNA damage. Since defects in DNA replication are increasingly recognized as a major source of genomic instability, the "Maintenance of Genome Integrity during DNA Replication" group aims at identifying the origin of replication stress. Exploring how cancer cells respond to and tolerate DNA replication impediments are the objectives of the group "Genetic Instability and Cancer". Meanwhile, the team "Genome Surveillance and Stability" explores the molecular mechanisms by which checkpoint signals are generated in the presence of DNA lesions, particularly during early embryogenesis. Deciphering the transcriptional reprogramming induced by DNA lesions and the interplay between DNA repair and innate immune response are aims of the "Molecular Virology" team. Finally, understanding the physical and functional interactions between cell cycle regulators and the DNA damage response is the aim of the "Microtubules and Cell Cycle" group.

Infectious diseases.
Infectious diseases are a major public health problem world-wide. HIV/AIDS constitutes one of the public health issues of the Millennium Development Goals. Understanding the intimate interaction between HIV and its host is an important challenge which, if achieved, may lead to the development of effective therapies and/or a vaccine. Major efforts in the department are channeled towards a better understanding of the physical and functional interactions between HIV and the immune system, particularly its co-receptors (CCR5 and CXCR4), which results in immune activation. This is the main objective of the team "Homing, Immune Activation and Infection". Moreover, improving our understanding of the interaction between HIV and its host with particular emphasis on HIV persistence and host resistance are the major aims of the "Molecular Virology" group.

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IGH

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Molecular Virology

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Human Immunodeficiency Virus type 1 (HIV-1), the causative agent of AIDS, is a retrovirus that primarily infects cells of the immune system. The outcome of HIV-1 infection is the result of complex interactions between viral proteins and host cell factors. In most cases, HIV-1 successfully hijacks cellular pathways and bypasses cellular restriction factors for optimal replication, leading to continuous rounds of infection, replication and cell death. Ongoing viral replication causes the loss of CD4+ T cells and progression to immunodeficiency in infected individuals. Major advances in HIV/AIDS treatment regimens have fundamentally altered the natural history of the disease and sharply reduced HIV-related morbidity and mortality in countries where such treatments are accessible. The most notable advance is the use of combination antiretroviral therapy or ART. However, after 15 years of treatment it is clear that ART is unable to achieve complete virus eradication or "sterilizing cure". Indeed, in most if not all cases, viral rebound is observed rapidly after ART interruption. Thus, life-long treatment is currently needed to control HIV. Drug resistance, cumulative side effects and high cost, represent major drawbacks of such treatments. Moreover, residual harmful inflammation and accelerated immune aging is observed even under optimized ART regimens. The persistence of HIV in treated patients results from the establishment of a viral reservoir insensitive to ART and poorly visible to the immune system. Thus, understanding HIV persistence and developing drugs able to flush out HIV, in order to achieve viral eradication or to decrease the need for continuous ART remain outstanding challenges. Our main objectives are to understand the complex interaction between HIV-1 and its host leading to viral persistence and escape from immune sensing. We are particularly interested in deciphering the molecular mechanisms involved in the regulation of HIV-1 gene expression and the role of host restriction factors in innate immune sensing of HIV.

1- Understanding HIV-1 gene expression through the identification of key regulatory host factors involved in activating or repressing the viral promoter.

Studying the HIV-1 transcriptional activator Tat has led to important progress in our understanding of transcription elongation by RNAPII, a key regulatory step of gene expression. To gain insight into the regulation of transcription elongation, we purified HIV-1 Tat-associated factors from HeLa nuclear extracts. We found that HIV-1 Tat assembles a multifunctional transcription elongation complex, which consists of the core active P-TEFb, MLL-fusion partners involved in leukemia (AF9, AFF4, AFF1, ENL and ELL) and PAF1/CDC73. Importantly, Tatcom1 formation relies on Cyclin T1 and CDK9, while optimal CDK9 CTD-kinase activity depends on the presence of AF9. Surprisingly, we found that Tat also associates and remodels the 7SK snRNP (Inactive PTEFb). Tat remodels 7SK snRNP by interacting directly with 7SKRNA in vivo, leading to the formation of stress-resistant 7SK snRNP particles (Sobhian et al. Mol Cell 2010). Besides the identification of new factors that are important for P-TEFb function and are required for Tat transcriptional activity, our data show a coordinated control of RNAPII elongation by different classes of transcription elongation factors acting at the same promoter.

More recently, in collaboration with Rosemary Kiernan's lab (IGH), we described a novel mechanism regulating RNAPII pausing and premature termination of transcription at the HIV-1 promoter. We found that microprocessor (consisting of Drosha and DGCR8) initiates premature termination by RNAPII at the HIV-1 promoter through cleavage of the stem-loop RNA, TAR and orchestrate the recruitment of the termination factors SETX and XRN2, and Rrp6 (Wagschal A. Rousset E. Basavarajaiah P. el al. Cell 2012).

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2- Understanding the crosstalk between HIV-1 replication and RNAi.

The rate of HIV-1 gene expression is a key step that determines the kinetics of virus spread and AIDS progression. Viral entry and gene expression are considered to be the key determinants for cell permissiveness to HIV. Recent reports highlighted the involvement of miRNAs in regulating HIV-1 replication post-transcriptionally (Triboulet et al. Science 2007). In this study we explored the role of cellular factors required for miRNA-mediated mRNA translational inhibition in regulating HIV-1 gene expression. We showed that HIV-1 mRNAs associate and co-localize with components of the RNA Induced Silencing Complex (RISC), and we characterized some of the proteins required for miRNA-mediated silencing (miRNA effectors). RCK/p54, GW182, LSm-1 and XRN1 negatively regulate HIV-1 gene expression by preventing viral mRNA association with polysomes. Interestingly, knockdown of RCK/p54 or DGCR8 resulted in virus reactivation in peripheral blood mononuclear cells (PBMCs) isolated from HIV-infected patients treated with suppressive HAART. microRNAs (miRNAs) are a class of small non-coding RNAs (sncRNAs) that function by regulating gene expression post-transcriptionally. Alterations in miRNA expression can dramatically influence cellular physiology and are associated with human diseases, including cancer. Here, we demonstrated cross-regulation between two components of the RNA interference machinery. Specific inhibition of Exportin-5, the karyopherin responsible for pre-miRNA export, down-regulates Dicer expression, the RNase III required for pre-miRNA maturation. This effect is post-transcriptional and results from increased nuclear localization of Dicer mRNA. In vitro assays and cellular RNA immunoprecipitation experiments showed that Exportin-5 directly interacts with Dicer mRNA. Titration of Exportin-5 by over-expressing either pre-miRNA or the adenoviral VA1 RNA resulted in loss of the Dicer mRNA/Exportin-5 interaction and reduction of Dicer level. This saturation also occurs during adenoviral infection and enhances viral replication. Our study reveals an important cross-regulatory mechanism between pre-miRNA or viral small RNAs and Dicer through XPO5 (Bennasser et al. Nat Struct Mol Biol. 2011).

3-Identification of host cell restriction factors.

In addition to the information required for the production of structural and enzymatic proteins essential for mature viral particles production, lentiviral genomes also encode auxiliary proteins that regulate viral fitness in hosts. Although these auxiliary proteins are mostly unnecessary for viral replication in permissive cells in vitro, disruption of open reading frames (ORFs) corresponding to individual viral auxiliary proteins results in inefficient viral spread ex vivo in non-permissive cells and in vivo in hosts. The primate lentivirus auxiliary protein Vpx counteracts an unknown restriction factor that renders human dendritic and myeloid cells largely refractory to HIV-1 infection. Here we identified Samhd1 as this restriction factor. Samhd1 is a protein involved in Aicardi-Goutière Syndrome (AGS), a genetic encephalopathy with symptoms mimicking congenital viral infections (Laguette et al. Nature 2011).

Eukaryotic organisms have been exposed to viral infections for millions of years. This co-evolutionary process has driven the development and adaptation of immune responses against invading viruses. In turn, viruses have evolved countermeasures to escape immune control. Through evolutionary studies, we found that SAMHD1 experienced strong positive selection episodes during primate evolution that occurred in the Catarrhini ancestral branch prior to the separation between hominoids (gibbons and great apes) and Old World monkeys. Importantly, we found that while SAMHD1 restriction activity towards HIV-1 is evolutionarily conserved, antagonism of SAMHD1 by Vpx is species-specific. The distinct evolutionary signature of SAMHD1 sheds light on the development of its antiviral specificity (Laguette et al Cell Host & Microbes 2011. Laguette and Benkirane. Trends immunology 2012).

4- NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function.

The Notch pathway is a master regulator of embryonic development and adult tissues homeostasis. Given its prominent role, dysfunctions and mutations in this pathway are associated with various human diseases including cancers. Despite important advances in our understanding of Notch signal transduction, the regulation of Notch functions in the nucleus remains unclear. Using immuno-affinity purification we identified NOTCH1 nuclear partners in T-ALL cells and showed that beyond the well characterized core activation complex (ICN1-CSL-MAML1) NOTCH1 assembles a multifunctional complex containing the transcription coactivator AF4p12, the PBAF nucleosome remodeling complex and the histone demethylases LSD1 and PHF8 acting through their demethylase activity to promote epigenetic modifications at Notch-target genes. Remarkably, LSD1 functions as a corepressor when associated with CSL-repressor complex and as a NOTCH1 coactivator upon Notch activation. Our work provides new insights into the molecular mechanisms that govern Notch transcriptional activity and represents the first glimpse into NOTCH1 interaction landscape, which will help deciphering mechanisms of NOTCH1 functions and regulation (Yatim et al. Mol Cell 2012).

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Genetic Instability and Cancer

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From the earliest stages of tumorigenesis, deregulated oncogenes perturb DNA replication, induce the formation of DNA double-strand breaks (DSBs) and activate DNA damage responses. Replication-associated defects can result from chemical alterations in the DNA template, from nucleotide pool imbalance and/or from conflicts between the replication and transcription machineries. Our goal is to unveil key mechanisms that are essential for cells to overcome replication impediments. We believe that these mechanisms are important determinants of tumor growth and resistance to chemotherapies.

To cope with DNA lesions and replication catastrophes, cells have evolved along with a sophisticated DNA damage response (DDR) that orchestrates the repair of DNA and the resolution of problems during DNA replication in coordination with ongoing physiological processes. A number of proteins necessary to implement this response are disabled in chromosomal instability and cancer prone disorders.

1. Elucidation of the mechanisms implicated in the signaling of damaged replication intermediates

Proteins in the DNA damage response network are typically controlled via phosphorylation, ubiquitination or poly (ADP-Ribosyl)ation reactions, which impact on protein function, protein recruitment and protein turnover. We are using human cell free extracts to identify DNA structural features and molecular mechanisms that are implicated in the nucleation of DNA damage signaling complexes.

For instance, we found recently that the juxtaposition of a double-stranded DNA end and a short single-stranded DNA gap can trigger robust activation of endogenous ATR and Chk1 in human cell-free extracts. This DNA damage signal depended on DNA-PKcs and ATR, which congregated onto gapped linear duplex DNA. DNA-PKcs primed ATR/Chk1 activation through DNA structure-specific phosphorylation of RPA32 and TopBP1. The synergistic activation of DNA-PKcs and ATR suggests that the two kinases can combine to form a signalosome implicated in a prompt and specific response to replication-born DSBs.

2. Exploration of the molecular function of FANC and FANC - associated proteins.

Studies of the rare genetic disease Fanconi anemia provide important knowledge on how cells respond to endogenous replication obstacles, on the nature of these obstacles, and on how cells can surmount chemotherapeutic treatments that cause replication failure. Hence, a major focus in the laboratory is on Fanconi anemia proteins, which function as an integration hub in the cellular responses to DNA replication stress.

We obtained recently novel insights into the tumor suppression function of FANCD2, a key effector protein in the Fanconi anemia / BRCA network. We found that FANCD2 and FANCI bind newly synthesized DNA in response to replication impediments. FANCD2 targeted stalled forks via an association with the minichromosome maintenance (MCM) replicative helicase. Using DNA fiber labeling for the visualization of replication tracts at the single molecule level, we observed that FANCD2 was necessary to actively arrest replication forks that are ongoing in the presence of a reduced pool of nucleotides. In human primary cells,

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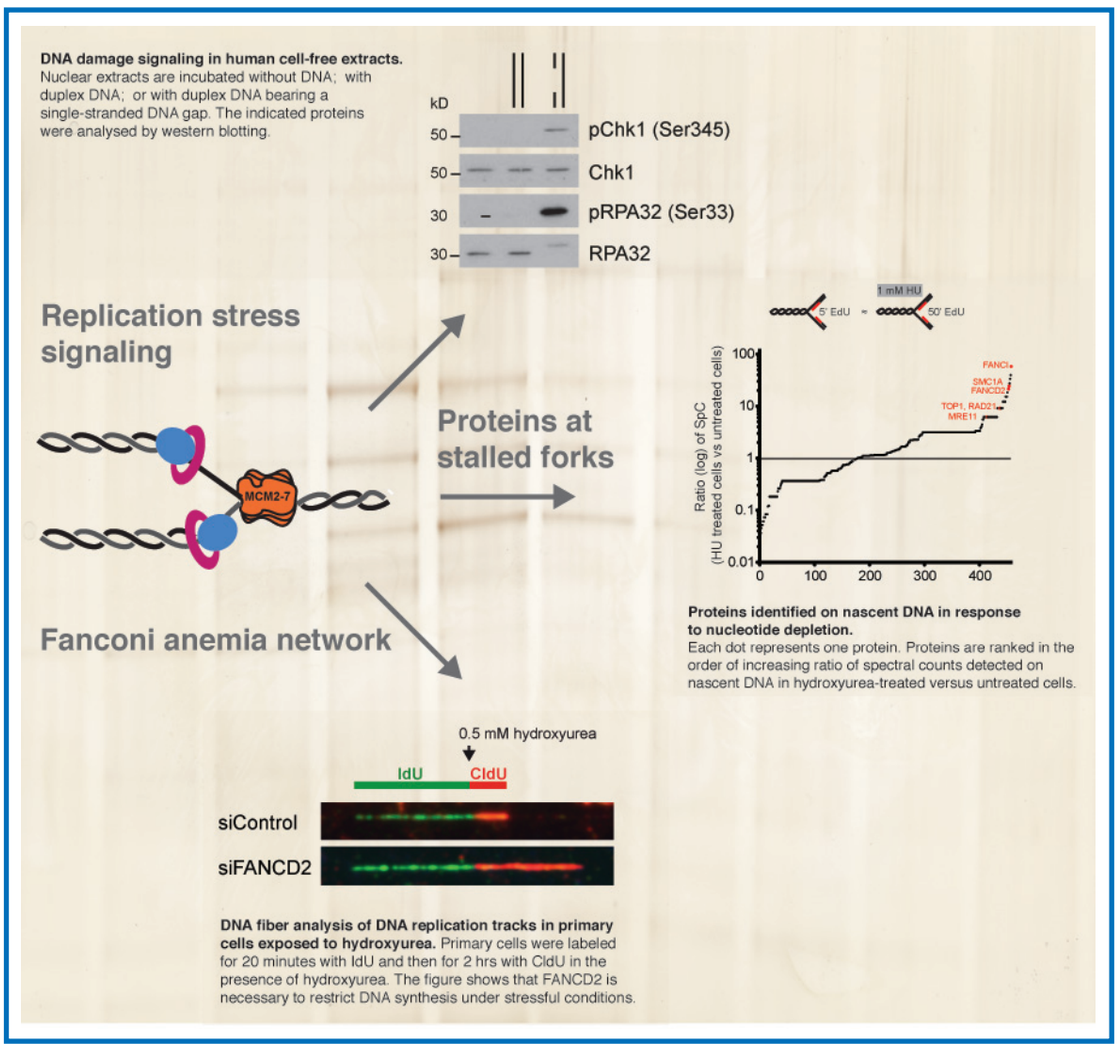
FANCD2 prevented the accumulation of replication-associated lesions, the induction of p21, and the entry of cells into senescence. We believe that FANCD2 is an effector of ATR signaling implicated in a general replisome surveillance mechanism that is necessary to sustain cell proliferation and attenuate carcinogenesis.

3. Biochemical characterization of stalled replisomes

To unveil novel replication stress tolerance mechanisms, we are purifying and identifying systematically proteins bound to newly synthesized DNA in the vicinity of stalled replication forks. We explore the function of novel factors identified at stalled forks using a variety of biochemical and cell biological approaches.



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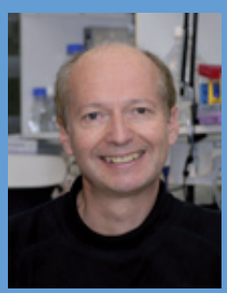

Homing, Immune Activation and Infection

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Our research interests are focused on the roles played by the chemokine receptors CCR5 and CXCR4 and by immune activation in Human Immunodeficiency Virus type 1 (HIV-1) infection. CCR5 is used as a coreceptor in addition to CD4 by the vast majority of HIV-1 virions ("R5 strains"), whereas CXCR4-using ("X4 strains") HIV-1 strains emerge eventually in some infected individuals, preferentially at later stages of the disease. A distinctive feature of our team is to conduct basic and clinical research at the same time.

We have previously shown that:

- the level of CCR5 and CXCR4 expression at the surface of CD4+ T lymphocytes drastically determines the level of productive infection of these cells by the R5 and X4 strains, respectively
- CCR5 and CXCR4 are used by the virus not only to bind to the target cell but also to activate this cell in order to optimize its own replication
- two CXCR4 isoforms are coexpressed in Humans, A and B; both have the same activity as chemokine receptors, but the B isoform is much more efficient as a coreceptor for X4 strains; moreover, R5 infection favors CXCR4-B expression over that of CXCR4-A, hastening the emergence of X4 strains.

We are currently working on two research themes.

1 - Identification of G protein-coupled receptors that interfere with CCR5 function. Some G protein-coupled receptors (GPCR) heterodimerize and this heterodimerization may modify their capacity to bind to ligands and/or the signaling triggered as a consequence of this binding. We have identified GPCR that heterodimerize with CCR5 at the surface of CD4+ T lymphocytes. Some of these GPCR modify the function of CCR5 as an HIV coreceptor. We are studying the mechanisms responsible for these modifications. We are also looking for the effect on HIV infection of specific ligands of these GPCR.

2 – Immune activation in the course of HIV infection. A hallmark of HIV infection is the number and diversity of the immune cells that it activates, even in patients under highly active antiretroviral therapies (HAART). The persistence of residual immune activation favours immune deficiency and various morbidities, including atherothrombosis, osteoporosis, neurocognitive disorders, liver steatosis, and some types of cancer. We are studying in patients aviremic under HAART the causes, the phenotypes and the consequences of immune activation. Our working hypothesis is that various patterns of immune activation might be identified in these patients. Each pattern might be the consequence of specific causes of immune activation, and might result in specific pathogenic consequences.

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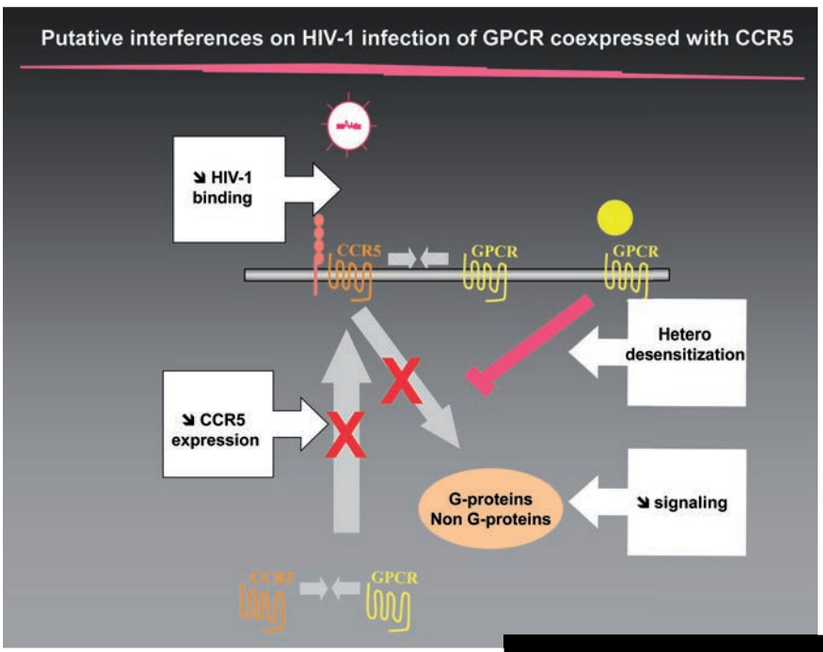

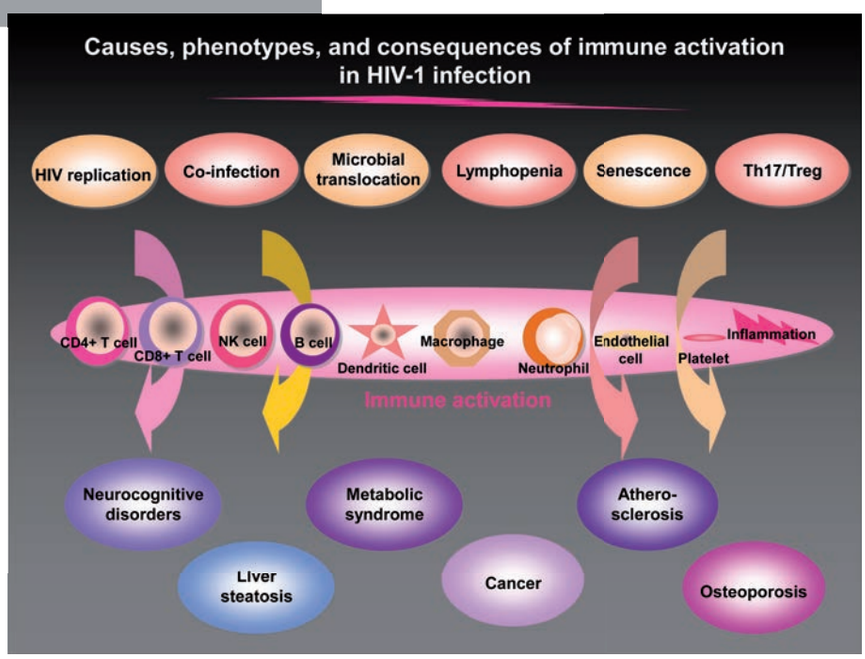

IMGT® - the international ImMunoGeneTics information system®

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Our research activities are focused on molecular immunogenetics, immunoinformatics, bioinformatics and rare genetic diseases. We are studying the genetics, structures, functions and repertoires of the immunoglobulins (IG) of B lymphocytes and plasmocytes, and of the T cell receptors (TR) on T lymphocytes, which are essential components of the adaptive immunity in humans and other vertebrates.

In 1989, IMGT®, the international ImMunoGeneTics information system®, which is at the birth of immunoinformatics, was created by Marie-Paule Lefranc (Montpellier 2 University and CNRS). IMGT® is the global reference in immunogenetics and immunoinformatics. IMGT® is a CNRS registered trademark (EU, Canada and USA). IMGT® is certified ISO 9001:2008 since 2010 (renewed in 2013) and NFX 50-900 since 2014.

IMGT® is specialized in the IG, TR and major histocompatibility (MH) proteins of vertebrates, and in the immunoglobulin superfamily (IgSF), MH superfamily (MhSF) and related proteins of the immune system (RPI). IMGT® is a high-quality integrated knowledge resource which provides a common access to expertly annotated genes, sequences and structures. IMGT® includes seven databases (IMGT/LIGM-DB, a comprehensive database of more than 176,000 IG and TR sequences from 346 species in November 2014; IMGT/GENE-DB, IMGT/CLL-DB, IMGT/PRIMER-DB, IMGT/2Dstructure-DB, IMGT/3Dstructure-DB and IMGT/mAb-DB), seventeen interactive tools and more than 15,000 pages of Web resources. IMGT/DomainGapAlign is widely used for antibody engineering and design of humanized antibodies as it allows the precise definition of FR-IMGT and CDR-IMGT and the easy comparison of amino acid sequences between the nonhuman (mouse, rat…) V domains and the closest human germline genes. IMGT/HighV-QUEST, the only online portal for IG and TR Next Generation Sequencing (NGS) data, has analysed more than 3 billions of IG and TR sequences in 2014, from 998 users (45% USA, 36% Europe, 19% other countries).

Since July 1995, IMGT® is available on the Web at http://www.imgt.org. IMGT® is used by academic and industrial scientists involved in fundamental research, medical research (autoimmune and infectious diseases, AIDS, leukemia, lymphoma, myeloma), veterinary research, genomics (genome diversity and evolution of the adaptive immune system), biotechnology related to antibody engineering for humanization of therapeutic antibodies, diagnostics (detection of minimal residual diseases) and therapeutic approaches (grafts, immunotherapy, vaccinology). The IMGT® Web server at Montpellier is accessed by more than 80,000 sites per year. IMGT® has an exceptional response with more than 150,000 requests per month.

Antibodies represent a large number of the pharmaceutical substances submitted to the World Health Organization/International Nonproprietary Names (WHO/INN) Programme.

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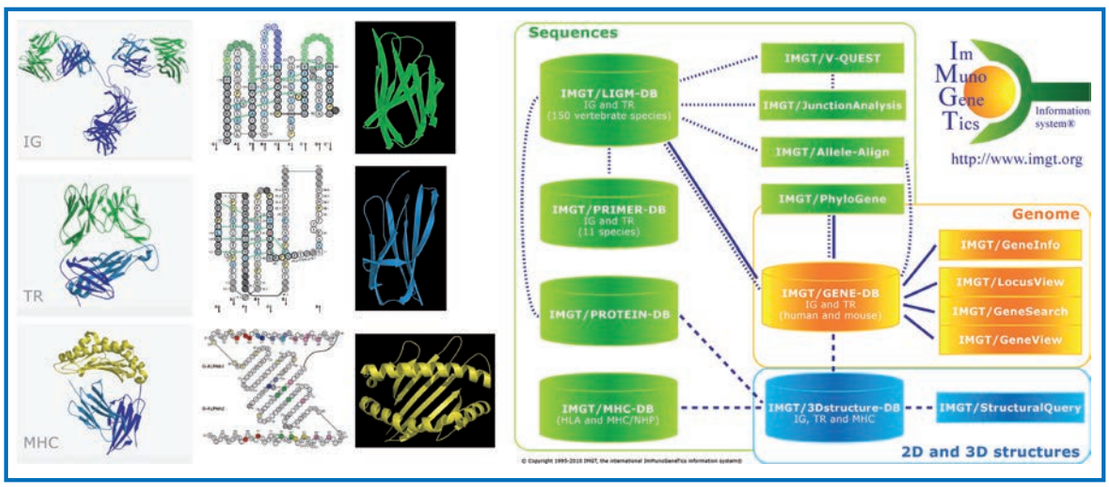

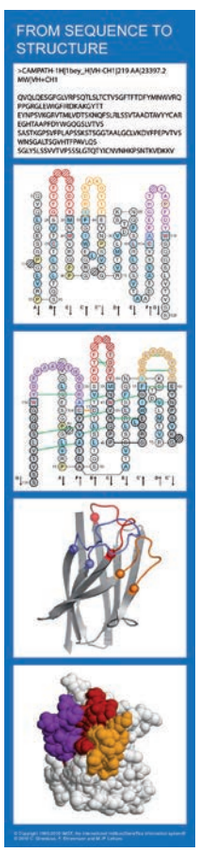

The INN definition of antibodies is based on the IMGT-ONTOLOGY concepts. Since 2008, amino acid sequences of monoclonal antibodies (mAb, INN suffix -mab), of fusion proteins for immune applications (FPIA, INN suffix -cept) and composite proteins for clinical applications (CPCA) from WHO/INN have been entered into IMGT®. These therapeutic applications emphasize the importance of the IMGT-ONTOLOGY concepts in bridging the gap between antibody sequences and 2D and 3D structures.

Another research interest, in collaboration mainly with the Unit of Medical Genetics, St-Joseph University, Beirut, and also with other teams in Tunisia and Algeria and the Children's Hospital of Boston (Pr Raif GEHA) concerns rare Immuno Deficiencies and autosomal recessive genetic diseases in consanguineous families (there are as many as 25% of marriages between cousins, often first cousins and even double-first). The patients are autozygous (homozygous by descent) for very rare mutated genes and haplotypes, present in the common ancestor(s) of their parents. These exceptional genotypes are invaluable starting points to allow the identification more quickly of the yet unknown mutated genes. Their functions in the cell organization or in signaling pathways, including the epigenetic and RNA silencing ones, are unmasked and can be investigated. The genetic counselling can be performed in these families.

The better understanding of the molecular basis of the pathophysiology allows better choices in the development of diagnostic tools and innovative therapeutics. This great improvement of knowledge is beneficial not only for the monogenic diseases, but also for the complex ones. Indeed, the consanguinity, responsible also for homozygosity of large chromosomal regions, identical by descent, allows to discover more easily the genetic networks. These statements are also valid for the search of genetic susceptibility or protection against infectious diseases.

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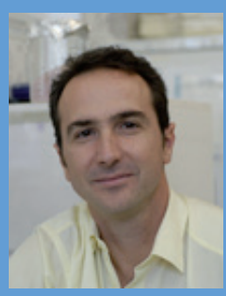

Genome Surveillance and Stability

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Our team is interested in the regulation of DNA damage checkpoints and DNA damage tolerance. These cellular processes are crucial for the maintenance of genomic stability when DNA integrity is compromised. Exposure to chemical compounds, replication fork arrest and endogenous cues, such as free oxygen radicals or the metabolism of the DNA itself, constitute major sources of mutations that continuously threaten the integrity of the cell genome. Checkpoint signals are generated in order to block cell division and activate repair pathways necessary to regenerate the normal DNA state. In the presence of high levels of damaged DNA this signaling pathway can promote the activation of programmed cell death, or apoptosis. DNA damage tolerance mechanisms that implicate translesion DNA synthesis also operate to rescue arrested replication forks thus avoiding fork collapse.

The experimental model systems employed are in vitro extracts derived from activated eggs of the amphibian Xenopus laevis as well as mammalian cells. Xenopus egg extracts faithfully reproduce the cell cycle in vitro and in particular the regulated activation of replication-independent and -dependent checkpoint signaling induced by different DNA damaging agents, such as UV rays, γ-radiations and genotoxic agents (cis-platin, methyl methanesulfonate).

Although genes controlling the DNA damage checkpoints are well conserved throughout evolution, a number of them are only found in vertebrates, and these are often mutated in several cancers. We have set up functional in vitro screens as well as in silico approaches to search for new, vertebrate-specific checkpoint genes and identified several candidates.

We are also interested in identifying the molecular mechanism of sensors activation, the proteins that recognize the lesions and, particular, the structures recognized by the sensors and the consequences of this recognition on the sensor functions. We have analyzed the specific role of the single stranded DNA binding protein RPA in S-phase checkpoint activation and surprisingly found that, in contrast to what generally admitted, its nucleation onto single stranded DNA generated at arrested forks is dispensable for checkpoint activation. We have also observed that in these conditions replication slows down and activates spontaneously the checkpoint through the production of single stranded DNA. Finally we have provided evidence suggesting that phosphorylation of the RPA2 subunit of the RPA complex is dispensable for checkpoint activation. These observations suggest that generation of single stranded DNA is a general cellular response to replication stress that functions in checkpoint activation independently of RPA. We have also characterized a novel factor required for checkpoint activation, a translesion DNA polymerase of the Y-family, Pol k. We have shown that in Xenopus this enzyme is implicated in formation of small replication intermediates produced onto single stranded DNA at arrested forks to facilitate recruitment of the checkpoint clamp 9-1-1 and promote Chk1 phosphorylation. We have also unveiled a novel mechanism by which TLS polymerases are recruited to UV damage sites, involving competitive interaction with PCNA orchestrated by the CRL4Cdt2 ubiquitin ligase.

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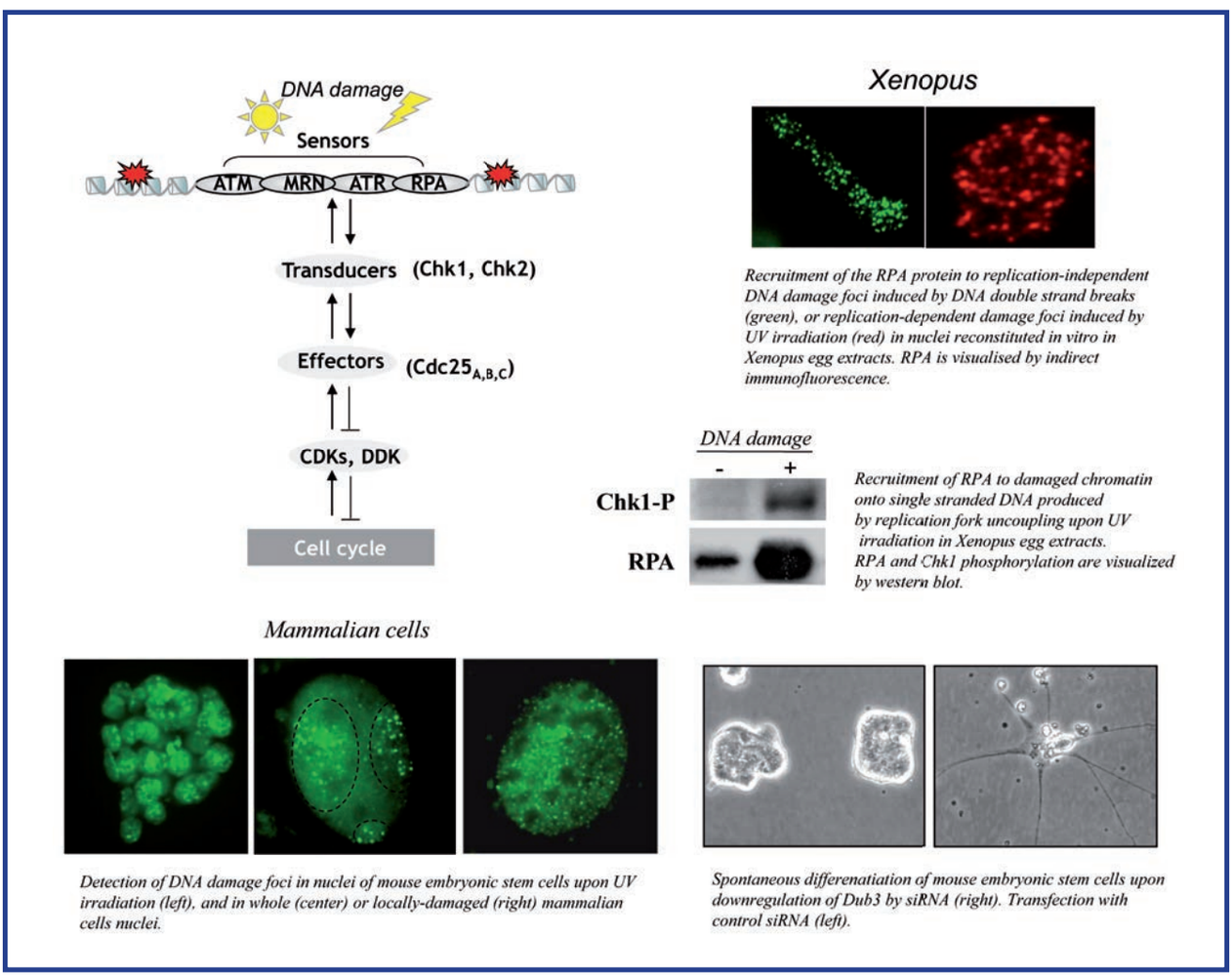
More recently we have explored the reasons of checkpoint inefficiency of mouse embryonic stem cells and shown that high levels of the Dub3 ubiquitin hydrolase sustain stabilization of the Cdc25A protein phosphatase, and by consequence G1/S checkpoint bypass upon UV damage. We have shown that this regulation is an intrinsic feature of pluripotent stem cells. We have observed that Dub3 is highly expressed in mouse embryonic stem cells since it is a target of two pluripotency transcription factors, Sox2 and Esrrβ, and that Dub3 is very rapidly down regulated upon differentiation, with faster kinetics than the well known pluripotency factor Oct4. Moreover, we have shown that donwregulation of Dub3 during differentiation is essential for cell viability, since forced expression of Dub3 induces massif cell death by interfering with cell cycle remodeling, while knockdown of Dub3 induces extensive heterogeneous differentiation. These features make of Dub3 a novel and highly specific marker of embryonic stem cells and strongly suggest that cell cycle remodeling is an essential feature of differentiation. Finally, we have observed that the expression of Dub3 and of several other pluripotency factors is cell cycle-regulated in mouse embryonic stem cells. **For more information see the team web page: http://www.igh.cnrs.fr/equip/domenico.maiorano/**



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Maintenance of Genome Integrity during DNA Replication

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Genomic instability is an invariant property of cancer cells that is characterized with an increased rate of mutations and gross chromosome rearrangements. Spontaneous chromosome breaks are detected very early in the cancer process, as a consequence of oncogene-induced DNA replication stress. These DNA breaks lead to the constitutive activation of ATR/ATM-dependent checkpoint pathways and raise a barrier against tumor progression. According to the so-called "oncogene-induced DNA damage model of cancer development", loss of p53 allows precancerous cells to escape this barrier and to progress through the cancer process. This model is very attractive as it explains two key features of cancer: genomic instability and the high frequency of p53 mutations. However, the mechanism by which deregulated oncogene expression induces DNA replication defects remains largely unknown.

Replication stress represents a double-edge sword for cancer cells. Although it helps them accumulate mutations and escape anti-tumor barriers, it also impedes the duplication and the segregation of their chromosomes and makes them hypersensitive to genotoxic agents. This increased sensitivity to DNA damage represents the Achilles' heel of the tumor and is exploited in chemotherapy to target cancer cells. However, aggressive tumors often adapt to replication stress and escape treatment. Understanding how replication stress arises in precancerous lesions and how cancer cells deal with stalled and damaged forks to escape chemotherapy remains therefore a major challenge in cancer research.

DNA replication is a complex process that depends on the activation of thousands of origins distributed along the chromosomes. Origin activation follows a well-defined replication timing program that is imposed by the local chromosome environment. A large body of evidence indicates that the correct execution of this replication program is important for the maintenance of genome integrity. However, the molecular determinants of the replication timing program remain poorly characterized. Replication forks progressing bidirectionally from active origins frequently stall when they encounter obstacles such as DNA lesions or tightly-bound proteins complexes. Studies in model organisms have shown that stalled forks are fragile structures that must be promptly restarted to prevent the formation of DNA breaks and/or toxic recombination intermediates. Stalled forks can be rescued by forks progressing from dormant replication origins, which are normally silent but fire in replication stress conditions. In the absence of dormant origins, replication fork recovery depends on various mechanisms involving checkpoint kinases, specialized helicases and recombinational repair pathways.

The research conducted in our lab addresses three central questions at the interface between DNA replication, genomic instability and cancer:

(i) What are the molecular determinants of the DNA replication program?

(ii) What causes spontaneous replication stress in eukaryotic cells?

(iii) How do cells respond and adapt to replication stress?

We use yeast and human cell lines as model organisms to identify regions of the genome that are intrinsically difficult to replicate and that induce spontaneous replication stress. We also investigate the cellular responses to replication stress in normal cells and in cancer cell lines. To this end, we take advantage of powerful new technologies, such as DNA combing and ChIP-seq, to monitor origin firing and replication fork progression both at the single-molecule and genome-wide levels.

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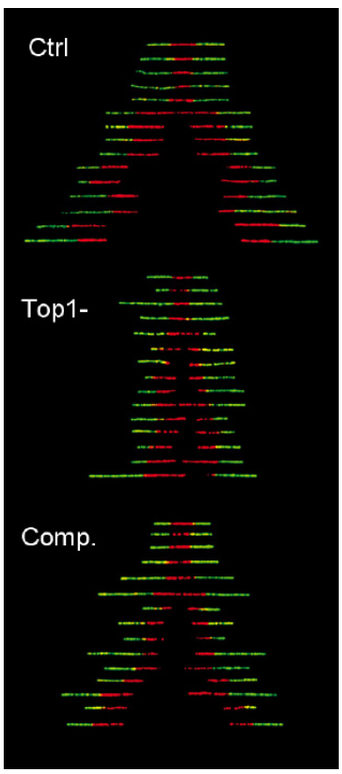
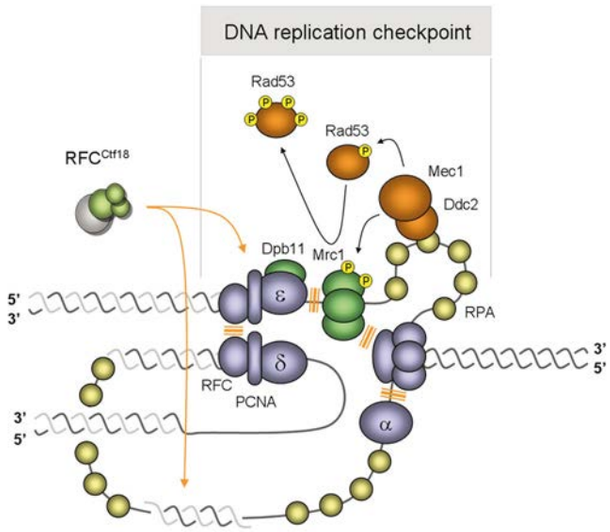
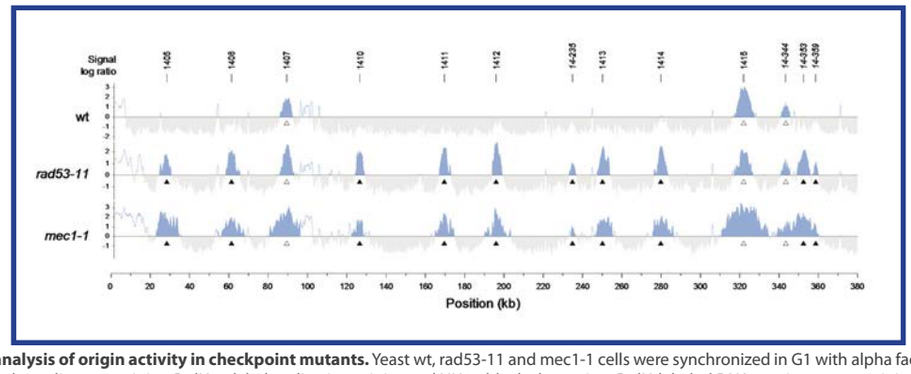


DNA combing analysis of replication forks progression and pausing in Top1-deficient mouse cells. Control mouse P388 cells (Ctrl), Top1-deficient cells (Top1-) and Top1-deficient cells complemented with human Top1 were analysed by DNA combing after two pulses of IdU (red) and CldU (green). Replication forks progress more slowly and pause more frequently in Top1- cells (Tuduri et al., 2010).



Activation of the DNA replication checkpoint in budding yeast. Accumulation of ssDNA at stalled forks is detected by the ATR-homolog Mec1, which activates the effector kinase Rad53. Amplification of the checkpoint response depends on the checkpoint mediator Mrc1. Recent evidence also indicate that the RFC-Ctf18 complex, best known for its role in the establishment of sister-chromatid cohesion, is also essential for the Mrc1-dependent activation of Rad53 (Crabbé et al., 2010).



BrdU-IP-chip analysis of origin activity in checkpoint mutants. Yeast wt, rad53-11 and mec1-1 cells were synchronized in G1 with alpha factor and were released for 90 min in fresh medium containing BrdU to label replication origins and HU to block elongation. BrdU-labeled DNA was immunoprecipitated and hybridized on Affymetrix tiling arrays. A map of a fraction of chromosome XIV is shown. Empty arrowheads: early origins. Filled arrowheads: late origins.

ADMINISTRATION

Administrator : Brigitte MANGONI - Brigitte.Mangoni@igh.cnrs.fr

- **Executive secretariat** : Anne-Pascale BOTONNET
- **Administrative secretariat** : Silke CONQUET
- **EpiGenMed secretariat** : Stéphanie MARTINETTI

Administrator : BRIGITTE MANGONI

The Administrator ensures, for the Unit Director, the administrative, financial and logistic coordination of the central services. She is delegated by the Director to coordinate and supervise the administrative and support service teams. She is responsible for ensuring that the rules and regulations and procedures are respected.

Main activities:

* To direct and coordinate the activities of the shared administration-management service and of the common services (stores/logistics).
* To define, implement and adapt the organization and running of these services in accordance with the missions, the objectives and the assigned human and material resources.
* To assist and advise her hierarchy concerning the budget preparation, the monitoring of the budget allocation and implementation, the application of the purchase policy, the coordination of the human resources and HR policy (recruitments, management of the unit personnel) as well as the implementation of the health and safety policy.
* To monitor that the health and safety rules and regulations are put in practice.
* To contribute to the IGH general administration, to sensitive and strategic issues (Laboratory committee, valorization of research results, 5-year review, budget requests, internal rules and regulations).
* To carry out the annual performance and development reviews with the administrative/management service employees.
* To represent the direction for interventions concerning the administration and management domains (INSB – CNRS Institute of Biological Sciences, CNRS and INSERM regional offices, Universities).
* To implement and monitor all management acts and procedures which are of her competence.
* To take part in administrative surveys.
* To prepare all the administrative documents concerning the unit staff and ensure the individualized follow-up of the staff administrative situation.
* To manage the contract staff (preparation and follow-up of the work contracts, to advise group leaders on the ad hoc nature of the recruitment).
* To inform, assist and advise the unit personnel and Director.
* To prepare the financial report, to analyze the expenses.
* The help in preparing the research contracts and agreements.
* To follow the research contracts.
* To manage the unit and site facilities (premises, security, cleaning, preparation of the work specifications).

FINANCIAL MANAGEMENT

Sahondra RAKOTONDRAMASY
Marie-Claire MERRIOT
Eric SMAGGHUE
Harizakanirina RAJAONARIVELO

- FINANCIAL MANAGEMENT OF THE LABORATORIES :
- Order forms (5000/year), invoices, travel, reimbursements (500/year), incomes, notifications;
- Agreements, equipment purchase and tenders;
- Help with the preparation of the budget, and follow-up of the budget implementation.

INSTITUTE OF HUMAN GENETIC

COMPUTING FACILITY

GUILLAUME GIELLY

Guillaume.Gielly@igh.cnrs.fr

Guillaume Gielly
Engineer CNRS

Jacques Faure,
Technician CNRS

Alfred Vriese,
Engineer CNRS

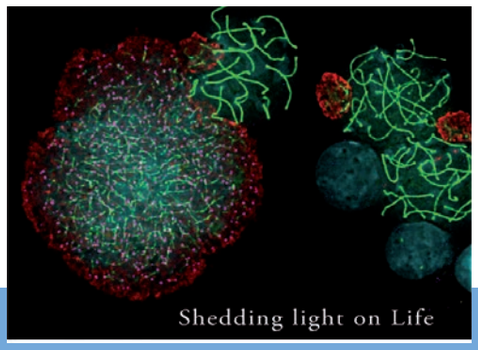
The The computing staff assures the smooth running of the computing facility (network infrastructures and services, database servers, grid computing servers, backup and virtualization servers), offers help and advice to the users at the Institute of Human Genetics (IGH) and is involved in IT research and development.

The computing facility includes three full-time employees who run the IT infrastructure, offer computational support and assure the technological monitoring. The different activities of the service include:

- The choice and daily installation of common resources for the exploitation systems and network software: DNS (Domain Name System), mail, anti-spam, web homepages, backup, virtualization, diffusion lists, users' directories, and compute server for data analysis. About one hundred physical and virtual servers are housed at the Institute.
- The management of the local server, the remote groups at the CHU and IURC sites as well as the security: +600 Ethernet sockets, definition and implementation of the security policy.
- Hosting the FTP mirrors: GNU & Savannah, Debian-Multimedia; the GNU/Linux and BSD (0linux, Nutyx, PC-BSD) distributions and the software forge for the free NetBSD project
- Users' support: advice, troubleshooting, training
- Development of innovative solutions to answer to specific users' needs.
- Purchase of IT equipment and software for the Institute after having taken into consideration the users' preferences and requirements
- Management of the IP telephony infrastructure
- Management of the groups' web servers and databases
- Software licensing
- Technological monitoring activity

Moreover, we are playing an active role in a new scientific facility (MAGMA: Make Analysis in Montpellier fAcilities) that offers the opportunity to the research groups in the Languedoc-Roussillon region of carrying out powerful analyses of sequencing data. A cluster system has been set up in partnership with the Institute of Functional Genomics (Institut de Génomique Fonctionnelle, IGF) in order to offer high speed access with high availability. An original data storage system (4U-high, 90To in ZFS) has been developed by the IGH computing staff to answer to the need of an important disk volume.
The computing service is also in charge of running the servers of the on line IFR3 library. This is a structure that groups together about 176 researchers and nine INSERM, CNRS, University and Hospital laboratories of Montpellier.

We host also several databases to make scientific data available to the scientific community.



INSTITUTE OF HUMAN GENETIC

CELL IMAGING FACILITY

JULIEN CAU

Julien.Cau@igh.cnrs.fr

Julien Cau
Engineer CNRS

Julio Mateos-Langerak
Engineer CNRS

Amélie Sarrazin
Engineer

The cell imaging facility of the Arnaud de Villeneuve/IFR3 campus is located on the basement floor of the Institute of Human Genetics. On its premises (approx. 100 m2) state-of-the-art image acquisition and analysis workstations are housed under the supervision of two-three scientific officers.
The facility is part of the Montpellier RIO Imaging distributed facility. This structure is dedicated to light/electron microscopy, X-ray tomography and flow cytometry. The facility is managed within an ISO:9001 framework (i.e., its main aims are increased users' satisfaction and continuous improvement). The whole facility is used by about 600 active users over the city and the site at the Institute of Human Genetics by approximatively 150 people.

The facility hosts the equipment previously located within the building (5 widefield microscopes). It also offers three confocal microscopes for high resolution observation of thick samples: a regular one, a macro-confocal (for observation of sample up to 19mm wide) and a high sensitivity set-up (with GaAsP detectors). The facility recently entered the super-resolution path following the acquisition of a structured illumination microscope. This piece of equipment, under the supervision of a dedicated engineer, allows the observation of specimens with a lateral resolution of 100nm and an axial resolution of 300nm. Thus the observation volume is 8 times smaller, allowing super-resolution imaging of samples. Images and the derived data from any workstation can be further analyzed on dedicated computers (deconvolution, 3D rendering, 3D image processing and measurements, analysis automation). The detailed list of the services provided by each workstation is included in the facility web site (see www.mri.cnrs.fr).

Beside this state-of-the-art equipment, a set of good quality microscopes (stereomicroscope, upright and inverted microscopes) are available on a free-access basis (no booking required) for rapid inspection of samples or sample preparation/ dissection at the laboratory bench.

New users are encouraged to contact the facility manager in order to have a brief introduction about the facility rules and to better identify their needs in cell imaging before they prepare their samples.

INFORMATIC DEVELOPMENT FOR RESEARCH SUPPORT

CYRIL SARRAUSTE de MENTHIERE

Cyril.Sarrauste@igh.cnrs.fr

Cyril Sarrauste
Engineer CNRS

Eric Stossel
Assistant
Engineer CNRS

The mission of the service is to design and develop programs or databases for "dynamic access" applications available on the web.

Our work involves the maintenance and development of the institute website with programs and intranet tools for both scientific and administrative operations within the institute. For example, these comprise an institute booking system for all common equipment, seamless updating of the institute publication database, various administrative directory services including the personnel directory, the research groups' directory and the secretarial and administrative staff's directory. In addition, thanks to these tools, the different services and group leaders can manage and update the databases and / or the information of their own web pages.
Concerning the development side, when a research group or department has specific projects with needs beyond the strict confines of the IGH, we analyze the project requirements to design, develop and implement tools both web-based and at the workstation level.

For example,
• TraCSEH: a traceability tool for human embryonic stem cells,
• WebCongress: a complete environment for managing the organization of seminars up to international conferences, ranging from speakers' registration, abstract submission and review to automatic badge generation, abstract book production and the management of room assignment and billing.
• EpiGeneSys: tools tailored to the management of European project (7th FP) coordinated by IGH scientists.
• BioCampus Montpellier network of technological platforms for life sciences in Montpellier.
• Labex EPIGENMED a BioHealth research program selected by the French Ministry of Research and Education in the framework of the "Laboratoires d'Excellence" initiative.

The relevance of many of these tools, which have been specifically developed initially for the IGH (in particular WebCongress), is shown by their deployment now by regional and national institutions for their specific and own uses.
The facility also develops and supervises special projects for external laboratories, for instance:
• Design of the RHEM Website for the Network of Experimental Histology in Montpellier.
• Conception of the Genopolys (hub between researchers, clinicians, industries and publics) website and management tools.
• Management of the Hit Hidden HIV website: project funded by the European Commission, involving 5 organizations as IGH, the Pasteur Institute, the University of Ulm, the Centre hospitalier universitaire vaudois and Pharis Biotec GmbH, a german firm.
Finally, the service provides full user's support for all desktop softwares, bibliographic management tools, computer aided design (CAD) and computer aided publication (CAP).

Keywords: programming, databases, interfaces, bioinformatics, DAO

I G H
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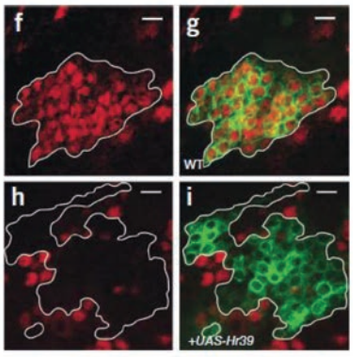
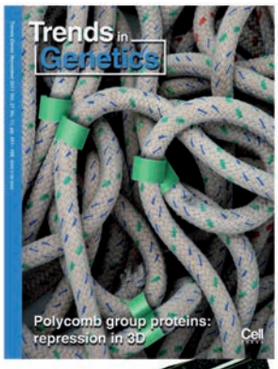
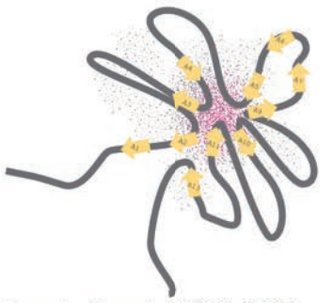
- C. Cartier, C. Sarrauste de Menthière inventors. (2009). Patentee: CNRS. "TraCSEH: Tool for Traceability of Human Embryonic Stem Cells". IDDN: FR.001.090013.001.SP2007.000.10000
- Milhavet F. Sarrauste de Menthière C., Touitou I. (2008). "The international society for systemic auto-inflammatory diseases (ISSAID)". **Clinical and Experimental Rheumatology,** 26, 222 DD1
- Milhavet, F. Cuisset, L., Hoffman, H., Slim, R., El-Shanti, H., Aksentijevich, I., Lesage, S., Waterham, H., Wise, C., Sarrauste de Menthière , C., Touitou, I. (2008). "The auto-inflammatory Infevers mutation online registry: update with new genes and functions". **HUM. TRANSFER**, 29, 803-808.

Some user's support in CAP / CAD



Published in Boulanger *et al.* Nat Neurosci. 2010





Trends Genet. 27(11) 2011 cover and published in Bantignies *et al.*

Cover of Cell 148(3) 2012

Cover of EMBO J. 33(10) 2014

Some projects

Data flow poster, shown in Vienna, dec. 2011

EPIGENMED

BiOCAMPUS Montpellier

hit hidden HIV

genopolys

Some uses of the «WebCongresses» tool

COMMUNICATION & TRAINING PROGRAM

CATHERINE LAROSE

Catherine.Larose@igh.cnrs.fr

The Communication department of the Institute serves as an interface between various audiences:
- internally, to facilitate the scientists, Institute and IGH staff interactions;
- externally, to connect the scientists and the Institute with different groups (e.g., citizens, decision makers, associations ...).

The IGH Communication Department co-operates with the Communication Department at the CNRS regional office (DR 13)

These actions aim at:
- Increasing the visibility of the Institute,
- Informing the scientific community on the scientific life of the Institute
- Informing the public about the activities of our Institute

The department contributes to both the internal and external IGH / CNRS communication and harmonizes projects with our partners.

We have focused our work specifically on:
- Organizational support for international meetings organized by IGH scientists on different topics, such as Epigenetics and Meiosis (2009), Conference Series on Nuclear Structure and Dynamics (2011, 2013)
- Development of relationships between academic institutions and scientists (Fête de la Science ...)

Institutions involved:
- DR 13 (CNRS regional office)
- CNRS communication department
- ADR 8 / INSERM (National)
- Universities 1 and 2 of Montpellier

Its missions include:
- Monitoring the implementation of the IGH science policy
- Relationship with the CNRS communication department and with other research institutes to facilitate the organization of events of scientific interest, especially directed towards young people (Fête de la Science ...) at the regional level.
- Preparation of scientific information to be used for communication, working closely with the IGH management
- The multidisciplinary perspective of scientific information.

IGH TRAINING CONTACT:

The CNRS employees continuous education is one of the main axes of the Institute human resource development policy. It focuses on the organization scientific priorities and on meeting the needs of skill development at the CNRS.

The instruments: The Unit Training Plan (PFU) is a written document that accompanies the collective discussion about a scientific project (or a project for a service) and about the skills required to support this project that is put in place by the CNRS Regional Office.

The recipients: Training courses financed by the CNRS are open only to the CNRS personnel and staff members paid by the CNRS (permanent staff, trainees, employees with a fixed term contract, associated researchers, PhD students and temporary staff). Non-CNRS employees who work in a CNRS unit may access training courses financed by the CNRS in the framework of their Unit Training Plan.

TECHNICAL SUPPORT

Health & Safety : Robert Orti

The health and safety engineer (ACMO) plans, implements and coordinates the institute safety programs to prevent and correct unsafe environmental working conditions

Technical Servicing : Daniel Bellenoue

Store : Faiza Laachir - Audrey Combe-Sainseau

The IGH stores contribute to the smooth running of the institute research activities and therefore improve the life of the IGH staff. Products and materials required by the research groups and the common facilities are available. The stock composition is mainly organized based on the researchers' requirements and proposals.

The catalog contains 1600 references.

Washing/Sterilization Service & Preparation of Laboratory Media

Scientific Leader : Armelle Lengronne
- Marie-Thérèse Molinier
- Samuel Crémier

Drosophila Facility

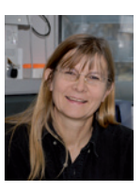
Scientific manager : Martine Simonelig
Manager : Bruno Mugat
- Stéphanie Chalmeton
- Mustapha Hanyn
- Fabienne Mazur

Animal Housing Facilities

Scientific Manager : Anne Fernandez
Manager : FLorence Arnal

Protected Zone

Scientific manager : Frédéric Baudat
Manager : FLorence Arnal



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ANIMAL HOUSING FACILITY PROTECTED ZONE

Our animal facility is a common infrastructure that is part of the IFR3.

Barrier Unit
Animal Housing Facility

Scientific manager
Anne Fernandez
Manager : Florence Arnal

PROTECTED ZONE
Scientific leader:
Frédéric Baudat
Manager : Florence Arnal

- Dominique Haddou

Microbiological status and hosted species:
- 140 m2 dedicated to the breeding and housing of genetically modified mice, under a specific pathogen-free (SPF) status. The entry into this SPF zone is strictly limited to the zootechnicians who take care of the animals. It is located in the IGH building and hosts about 6 000 mice permanently. 15 000 new animals per year are tagged for 22 user teams. The genotyping service spares tedious and time-consuming bench work for researchers, and ensures the timely delivery of genotype identification to the personnel taking care of the animals.

- 30 m2 for housing rabbits and Xenopus frogs under a conventional status. This zone, located in the IGH building, hosts rabbits used for the production of antibodies against specific epitopes, and Xenopus frogs to produce ovocytes for developmental biology or for the study of ionic channels.

- 60 m2 for rodents under a conventional status, in the IGF building. This facility hosts wild type mice and rats, and is also dedicated to short-time housing of class I genetically modified animals in view of quick testing of well-defined scientific hypotheses (promising mouse lines are then decontaminated and transferred into the SPF zone for long-term research projects). Moreover, the facility also provides help to researchers with injection protocols or small surgery (orchydectomy, ovariectomy...). We recently established an Ethics Committee for animal experimentation. Affiliated with the Ethics Committee of the Languedoc Roussillon region (CEEA-LR), this local committee is devoted to provide advice for designing experiments with animals and filling in the protocol forms to be submitted to the CEEA-LR.

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DROSOPHILA FACILITY

Scientific manager :
Martine Simonelig

Manager :
Bruno Mugat

- Stéphanie Chalmeton
- Mustapha Hanyn
- Fabienne Mazur

The IGH fly facility is a state-of-the-art fly-pushing and genetic manipulation service where all fly laboratories can grow flies, perform genetic and developmental biology experiments and maintain their stocks.

In terms of space, the facility has three rooms at different temperatures (18°C, 21°C and 25°C) and several high-precision incubators. Two more rooms are dedicated to the work with binocular microscopes, with 15 workstations equipped with CO2. A GFP-binocular is also available, as well as injection equipment for production of transgenic fly lines.

The facility personnel are in charge of maintaining the *Drosophila* laboratory stocks for each *Drosophila* group at the IGH. In total about 3.500 different *Drosophila* stocks are maintained permanently.

Furthermore, the *Drosophila* facility provides fly food to the whole Montpellier *Drosophila* community spread over four different institutes. The facility produces 10.000 ready-to-use *Drosophila* vials per week. As such, the services provided by the facility personnel are essential to the whole *Drosophila* community in Montpellier.

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SEMINAR SPEAKERS

PUBLICATIONS

2013

JANUARY

22-01-2013
Vanja Cankovic
The role of CTCFL/BORIS in meiosis

25-01-2013
Gaëlle Legube (Université Paul Sabatier- CNRS UMR 5088, France)
Transcription channels DNA double strand breaks to a RAD51-dependent repair pathway

28-01-2013
Satish SATI (SSH, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India)
Role of epigenetic modifications in maintaining tissue specific gene expression

FEBRUARY

01-02-2013
Jean-Yves Roignant (Institute of Molecular Biology gGmbH - Mainz - Germany)
Role of the exon junction complex in pre-mRNA splicing

05-02-2013
Bénédicte DURAND (Centre de Génétique et de Physiologie Moléculaire et Cellulaire - CGφMC UMR 5534 VILLEURBANNE)
From RFX transcription factors to cilia assembly: what can we learn?

08-02-2013
Crisanto Gutierrez (Centro de Biologia Molecular Severo Ochoa, Madrid, Spain)
Links of DNA replication and epigenetics: lessons from Arabidopsis

11-02-2013
Luciano Di Croce (Center for Genomic Regulation, Barcelona, Spain)
Role of chromatin structure and Polycomb complexes in embryonic stem cell differentiation

15-02-2013
Pierre-Yves Plaçais (GDSM, Laboratoire de Neurobiologie ESPCI - PARIS)
The hungry fly's brain disables costly long-term memory to favor survival

20-02-2013
Sophie KOSSIDA (Biomedical Research Foundation Academy of Athens)
Adventures of a bioinformatician over the last 15 years

MARCH

06-03-2013
Christian FELLER (Ludwig Maximilian University of Munich, Allemagne)
Chromatin3d: Topology andChromatin Modifications of a Co-Regulated Nuclear Domain

08-03-2013
Henri-Marc Bourbon (Centre de Biologie du Développement, Université Paul Sabatier TOULOUSE)
Transcriptional control of cell fate specification by Mediator complex subunits

15-03-2013
Nicolas Charlet-Berguerand (IGBMC - ILLKIRCH)
microRNA and mRNA alterations in RNA gain of function diseases

20-03-2013
Ozren Bogdanovic (Centro Andaluz de Biología del Desarrollo (CABD))
Deconstructing Repression: Integration of -omics approaches to understand developmental 5mC silencing pathways

29-03-2013
Vincent MOULY (Institut de Myologie, PARIS)
Regenerative capacity of human satellite cells

APRIL
02-04-2013
Frank Uhlmann (Cancer Research UK, London)
Establishment of sister chromatid cohesion during DNA replication

05-04-2013
Triantafyllos Gkikopoulos (University of Dundee, UK)
Set the controls for the heart of chromatin, DNA dependent and independent pathways in S. cerevisiae

12-04-2013
Thomas SURREY (London Research Institute UK)
Mechanistic insight into dynamic microtubule cytoskeleton functioning from cell-free fluorescence microscopy assays

16-04-2013
Alain NICOLAS (Institut CURIE Paris)
Roles of G-quadruplexes in genome instability

MAY
03-05-2013
Claude DESPLAN (Dept of Biology - New York University - USA)
Patterning the visual system. Stochastic vs. deterministic choices

17-05-2013
Ilan DAVIS (Dept Biochemistry - University of Oxford - UK)
The role of mRNA localisation and translational regulation in synaptic plasticity at the Drosophila neuromuscular junction

24-05-2013
Catherine DARGEMONT (Institut Jacques Monod - PARIS)
Ubiquitin conjugation: a timing mechanism for nuclear functions

JUNE
05-06-2013
Marta RADMAN-LIVAJA (IGMM Montpellier)
The heritability of chromatin configuration: a study in yeast

07-06-2013
Tatiana ALFONSO PEREZ (Cabimer, Séville)
Cytoplasmic interaction of the tumor suppressor protein hSNF5 with Dynamin-2 controls endocytosis

13-06-2013, 14h00
Marko Looke (University of Tartu, Estonia)
DNA replication initiation in budding yeast - the role of chromatin environment

17-06-2013
Jean DEUTSCH (Professeur Émérite Biologie du Développement, UMR 7622 Université P et M Curie, Paris 6)
What is a gene? The present crisis of the molecular concept of the gene

19-06-2013
Paulina Prorok (Institut Gustave Roussy)
The role of the human nucleotide incision repair in the removal of exocyclic DNA-base adducts and uracil from DNA

21-06-2013
Jorge BEIRA
On the edge: Regulation of Apoptosis Pathways Responsible for Tissue Homeostasis

21-06-2013
Klaus FÖRSTEMANN (Gene Center of the University of Munich, Germany)
siRNAs in genome defense against DNA damage and selfish genetic elements in Drosophila

28-06-2013
Eric MEYER (Institut de Biologie de l'Ecole Normale Supérieure CNRS UMR8197 - INSERM U1024 PARIS)
Transgenerational epigenetic inheritance of Paramecium mating types through co-optation of the scnRNA pathway

JULY
04-07-2013
Kerstin Bystricky (UPS, Laboratoire de Biologie Moleculaire Eucaryote, Universite de Toulouse)
Chromatin dynamics in transcription and repair

17-07-2013
Cécile Doyen
Chromatin modulators in paternal genome reprogramming

19-07-2013
Marc-Henri STERN (Institut Curie - PARIS)
The genetic landscape of uveal melanoma

19-07-2013
Margaret FULLER
Regulation of proliferation and differentiation in an adult stem cell Lineage

24-07-2013
Tom WANDLESS (Stanford University School of Medicine)
Tunable Control of Protein Stability using Small Molecules

26-07-2013
Karlene CIMPRICH (Stanford University School of Medicine)
Mechanisms for Maintaining Genome Stability at the Replication Fork

SEPTEMBER
16-09-2013
Antonin Morillon (Institut Curie, Paris)
Pervasive transcription, lessons from budding yeast

20-09-2013
Nathalie Dostatni (Professeur à l'UPMC UMR218 – CNRS & INSTITUT CURIE)
Transcriptional precision in the Bicoid system

20-09-2013
Blaise LI (Normale Sup, Paris)
Contributions to methods in phylogeny

OCTOBER
01-10-2013
Jean-René Huynh (Genetics and Developmental Biology - Institut Curie - Paris)
Protecting the genome and "pre-pairing" chromosomes for meiosis in Drosophila germ cells

08-10-2013
Atsuya NISHIYAMA (Nagoya City University, Japan)
Coupling DNA methylation to replication: the regulatory role of ubiquitin

16-10-2013
Sergei RAZIN (Russian Academy of Sciences)
New concepts in the 3D organization of the eukaryotic genome

NOVEMBER
08-11-2013
Douglas BISHOP (Cummings Life Science Center - University of Chicago - USA)
Architecture and Regulation of Meiotic Recombination Complexes

15-11-2013
Sébastien Britton (CNRS, Institut de Pharmacologie et de Biologie Structurale, Toulouse)
A new method for high-resolution imaging of Ku foci to decipher mechanisms and control of DNA double-strand break repair

22-11-2013
Michael WEGNER (Institut für Biochemie Universität Erlangen-Nürnberg, Germany)
Sox10 : a versatile regulator of vertebrate gliogenesis

25-11-2013Evi SOUTOGLOU (INSTITUT DE GENETIQUE DE BIOLOGIE MOLECULAIRE ET CELLURAIRE (IGBMC) - ILLKIRCH)
Nuclear compartmentalization and DNA repair

DECEMBER
04-12-2013
André VERDEL (Equipe ARN et Epigénétique Institut Albert Bonniot (IAB) - Grenoble)
RNA-degradation machineries and heterochromatin gene silencing in fission yeast

06-12-2013
Miguel FERREIRA (Telomere and Genome Stability Laboratory Instituto Gulbenkian de Ciência - OEIRAS-Portugal)
The role of telomerase in ageing and cancer

09-12-2013
Gérard ROIZES - Que nous apprennent les approches les plus avancées de la génétique moléculaire sur l'histoire des populations humaines ?

2013

11-12-2013
Marie-Claude BLATTER (Swiss Institute of Bioinformatics Geneva, Switzerland)
Protein sequence databases: use and pitfalls

16-12-2013
Jorge B. SCHVARTZMAN (Centro de Investigaciones Biológicas (CSIC), Madrid)
DNA topoisomerases are dispensable for the replication and segregation of yeast artificial chromosomes (YACs)

2014

JANUARY

10-01-2014
Jacques VAN HELDEN (Lab. Technologies Avancées pour la Génomique et la Clinique, INSERM U1090, Marseille, France)
Seminar Series on Genome Dynamics : Discovering motifs in massive sequence sets from NGS technologies

15-01-2014
Jean SOULIER (Hematology Laboratory Institut Universitaire d'Hématologie, University Paris Diderot Hôpital Saint-Louis, Paris)
Seminar series on Molecular Bases of Human Diseases: Pathogenesis of Fanconi anemia, a genomic instability disease with stem cell defect and cancer predisposition

15-01-2014,
Yves GAUDIN (Laboratoire de Virologie Moléculaire et Structurale, UPR 3296 CNRS, Gif sur Yvette)
Fusion mechanisms of enveloped viruses: what do we learn from rhabdoviruses?

16-01-2014
Philippe BENAROCH (Institut Curie, INSERM U932, PARIS)
A dynamic journey into HIV cycle in primary macrophages

17-01-2014
Eric MISKA (The Gurdon Institute, University of Cambridge, UK)
Seminar Series on Genome Dynamics : Transgenerational epigenetic inheritance and RNAe

24-01-2014
Constance CIAUDO (Inst. of Molecular Health Sciences, Zurich, Switzerland)
Seminar Series on Genome Dynamics : New small RNA functions in mouse embryonic stem cells

31-01-2014
Rob MARTIENSSEN (Cold Spring Harbor Laboratory, New York, USA)
Seminar Series on Genome Dynamics : Replication, Recombination, Repair and RNA interference coordinate heterochromatin in fission yeast

FEBRUARY

14-02-2014
Manolis PAPAMICHOS-CHRONAKIS (Dynamique nucléaire et plasticité du génome - Institu Curie - Paris)
INO80 chromatin remodeling links proteolysis to genome stability

21-02-2014
Pascal NOUVEL (Professeur de Philosophie UM3 - Montpellier)
Philosophie des Sciences

21-02-2014
Maria Elena TORRES-PADILLA (IGBMC, Illkirch, France)
Seminar Series on Genome Dynamics: Epigenetic mechanisms in early mammalian development

24-02-2014
Eileen FURLONG (EMBL, Genome Biology Department, Heidelberg, Germany)
Seminar Series on Genome Dynamics: Temporal properties of development enhancers in 3D

28-02-2014
Thomas JENUWEIN (Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany)
Seminar Series on Genome Dynamics: Genome-wide function of Suv39h-dependent H3K9me3

MARCH
04-03-2014
Chrysa LATRICK (Université de Nice Sophia Antipolis)
Tales from the telomere: A mechanism of increased telomerase processivity and the role of Rap1 in senescence

14-03-2014
Robin ALLSHIRE (Wellcome Trust Centre for Cell Biology, Edinburgh, UK)
Seminar Series on Genome Dynamics: Establishing and maintaining specialised chromatin domains

20-03-2014
Eskild LANDT (BRIC, University of Copenhagen)
Zbtb48 facilitates Polycomb Recruitment at CpG islands and modulates PRC2 activity in mouse embryonic stem cells

21-03-2014
Chris PONTING (Dept of Physiology,Anatomy and Genetics, Oxford University, UK)
Seminar Series on Genome Dynamics: Evolution and Cellular functions of long noncoding RNAs

APRIL
04-04-2014
Roser ZAURIN (CRG - Barcelona)
Changes in chromatin remodeling during hormone-dependent gene activation in Breast Cancer

08-04-2014
Yuki OGIYAMA (Osaka University)
Regulation of neocentromere formation in fission yeast

11-04-2014
Jason SWEDLOW (Centre for Gene Regulation & Expression College of Life Sciences University of Dundee)
Signalling and mechanics at the human mitotic centromere and kinetochore

11-04-2014
Laurent SCHAEFFER (Laboratoire Biologie Moléculaire de la Cellule, ENS, Lyon, France)
Seminar Series on Genetics & Development: Regulation of gene expression at the neuromuscular junction by electrical activity

18-04-2014
Alena SHKUMATAVA (Institut Curie - Unité de Génétique et Biologie du Développement - PARIS)
Seminar series on Genetics & Development: Conserved roles of lincRNAs in vertebrate development

24-04-2014
Alberto SPECK (Division of Infectious Diseases and Hospital Epidemiology University Hospital of Zurich)
Humanized mouse as model to study HIV pathogenesis

25-04-2014,
Mark WAINBERG (Centre SIDA McGill, Institut Lady David, Montréal, Canada)
Seminar series on Molecular bases of Human Diseases: What Is the Future of the HIV Epidemic If Drug Resistance against a New Antiviral Compound could not Occur?

25-04-2014
Kazufumi MOCHIZUKI (IMBA (Institute of Molecular Biotechnology of the Austrian Academy of Sciences) Vienna, Austria)
Seminar series on Genetics & Development: Small RNA-directed DNA elimination in Tetrahymena

MAY
14-05-2014
Vanessa F. BONAZZI (Centre Mediterraneen de Medecine Moleculaire, Nice)
Functional genetic in Melanoma

16-05-2014
Florence BESSE (Institut de Biologie Valrose, Nice, france)
Seminar series on Genetics & Development : Function and transport of RNP complexes in neuronal remodeling

22-05-2014
Bernadette BENSAUDE-VINCENT (Pr. de philosophie à l'université Paris 1)
Science et public

23-05-2014
Jan HOEIJMAKERS (Department of Genetics Erasmus University Medical Center - Rotterdam - The Netherlands)
Seminar series on Molecular bases of Human Diseases: DNA damage and repair: key factors for aging and cancer

JUNE
02-06-2014
Yégor VASSETZKY (Institut Gustave Roussy, Villejuif)
Nuclear organization and regulation of transcription in B-cell lymphomas and normal B-lymphocytes

06-06-2014
Marie-Christine CHABOISSIER (Institut de Biologie Valrose, Nice, France)
Seminar series on Genetics & Development : Male or female: R-spondin1 and sex determination

13-06-2014
Catherine RABOUILLE (Hubrecht Istitute, Utrecht, The Netherlands)
Seminar series on Genetics & Development: Coordinated response of secretion and RNA processing under aminoacid starvation

25-06-2014,
Yoav SOEN (Weizmann Institute of Science, Rehovot Israel)
Non-Mendelian mechanisms of inheritance of responses to toxic stress

26-06-2014
Anne ROYOU (Institut européen de chimie et biologie, IBGC - UMR 5095 Bordeaux)
Role and regulation of BubR1 during the segregation of broken chromosomes

30-06-2014
John MATTICK (Garvan Institute of Medical Research - Australia)
The human genome as the zip file extraordinaire

JULY
02-07-2014
Stanley NEUFELD (University of Calgary)
Functional interactions between Shox2 and Hox genes during the regional growth of the mouse limb

SEPTEMBER
05-09-2014
Shunichi TAKEDA (Department of Radiation Genetics Kyoto University)
Differential roles of double-strand break resection enzymes between yeast and vertebrates

12-09-2014
Alain ISRAEL (Institut Pasteur Paris)
The NF-kB signaling pathway: from molecules to pathologies

24-09-2014
Simone GILGENKRANTZ (Professeur émérite de génétique humaine, Université Henri Poincaré - Nancy 1)
Les débuts de la génétique en France

OCTOBER
06-10-2014
Karen ADELMAN, National Institute of Environmental Health Sciences,
Research Triangle Park, North Carolina, USA
Seminar Series on Genome Dynamics: New insights into mammalian gene regulation and immune responsiveness

15-10-2014, 11h30
Dirk HOSE (Myeloma laboratory at University Hospital of Heidelberg)
Multiple Myeloma: Molecular profiling, Pathogenesis, Targets & Translation – The way to a cure?

22-10-2014
Jim HABER
Genome stability and instability in the repair of chromosome breaks

28-10-2014
Nadine LAGUETTE (IGH Montpellier)
From HIV biology to Cancer Related Inflammation

29-10-2014
Manolis PAPAMICHOS-CHRONAKIS (Institut Curie - Paris (FRANCE))
Killing to survive: chromatin control of nuclear proteostasis

31-10-2014, 10h30
Kazufumi MOCHIZUKI (Institute of Molecular Biotechnology of the Austrian Academy of Sciences - Vienna Austria)
Gatekeepers for the RNAi machinery to enter the nucleus

NOVEMBER

04-11-2014, 10h30
Elzo DE WIT (Hubrecht Institute for Developmental Biology and Stem Cell Research - Utrecht (THE NETHERLANDS))
3D organization of the genome: fundamental principles and practical applications

06-11-2014, 11h30
Joan STEITZ (Yale University/HHMI, New Haven, USA)
Seminar series on Genome Dynamics: Noncoding RNAs: with a viral twist

07-11-2014, 11h30
Wouter de LAAT (Hubrecht Institute, Urtrecht, The Netherlands)
Seminar series on Genome Dynamics: Long-range gene regulation in the 3D genome: technologies and insight

10-11-2014
Germano CECERE (Columbia University Medical Center - New York, NY (USA))
Transcriptional and epigenetic regulation by short RNAs

12-11-2014
Ramesh PILLAI (European Molecular Biology Laboratory - Grenoble (FRANCE))
Genome defense by germline small RNAs

14-11-2014
Magdalena SKRZYPCZAK (Laboratory of Bioinformatics and Systems Biology CeNT University of Warsaw)
BLESS, Genome-wide high-resolution DNA double-strand break mapping

DECEMBER

02-12-2014
Marie-Claude BLATTER (Swiss Institute of Bioinformatics Geneva, Switzerland)
Protein sequence databases: use and pitfalls

19-12-2014
Robert SCHNEIDER (IGBMC Strasbourg)
Novel players in the regulation of chromatin function

2013

Akkouche, A., Grentzinger, T., Fablet, M., Armenise, C., Burlet, N., Braman, V., Chambeyron, S., Vieira, C. (2013) Maternally deposited germline piRNA silence the tirant retrotransposon in somatic cells. **EMBO Reports**, *14*, 5, 458-464. PMID : 23559065

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HOW TO FIND US

Plane:

Montpellier Méditérannée Airport about 3km to the South of Montpellier. (about 1/2 an hour from the IGH).

Train :

Montpellier SNCF train station - St Roch (downtown). The Bus Station is at the same place. (20 minutes away from the IGH).

Car:

* from A9 Toll highway, exit 29 Montpellier-Est (East) or exit 31 Montpellier-Ouest (West) : Follow North direction (20 minutes away from the IGH).

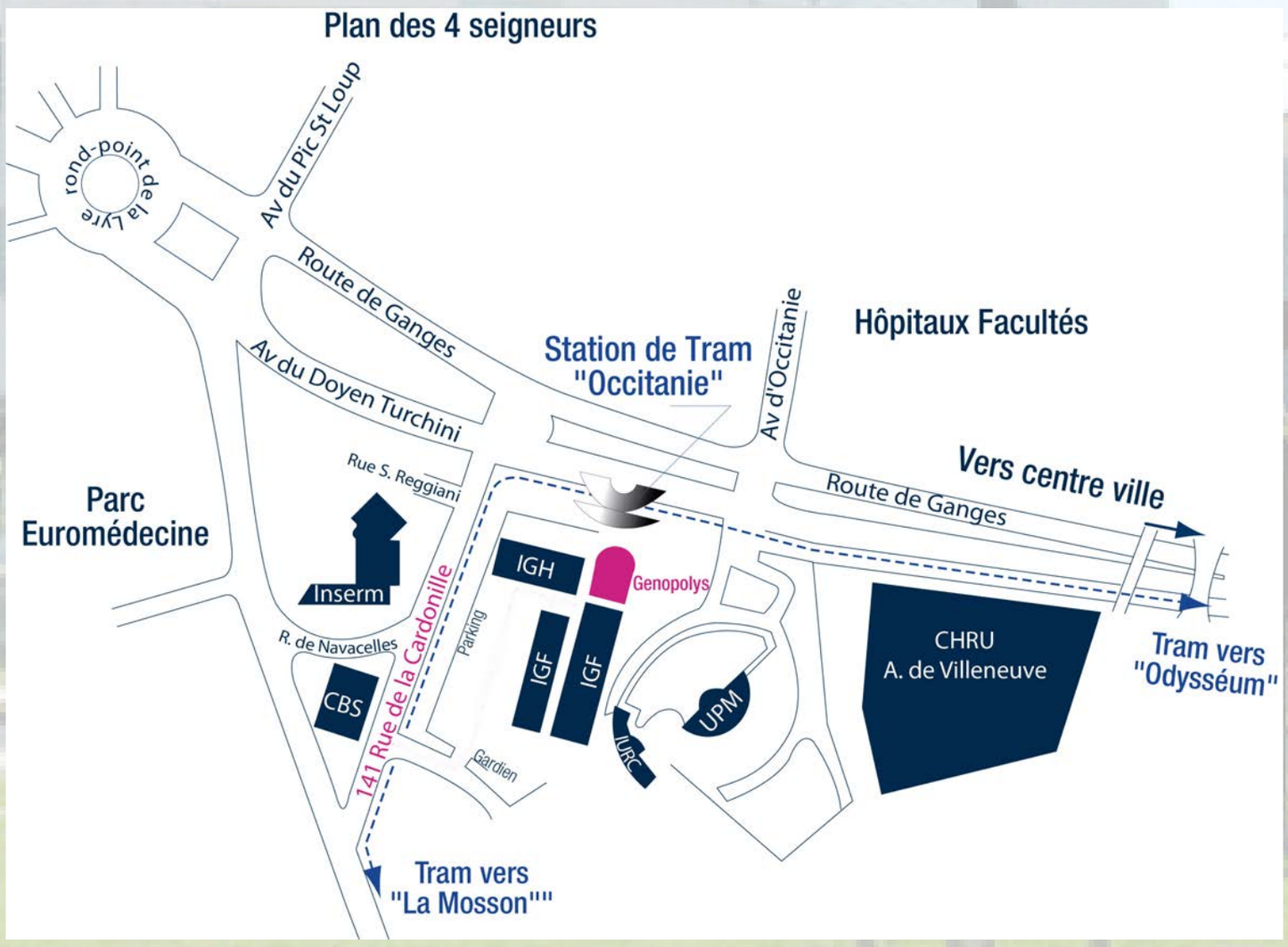
* from downtown : take the direction « Hôpitaux-Facultés » (10 minutes away from the IGH).

Bus-Tramway:

TAM network (From Downtown to the IGH) :

* Bus service N° 16 in the direction of "Euromédecine" get out at the "Occitanie" stop. about 25 min.

* Tramway service N° 1 in the direction of "Mosson" get out at the "Occitanie" stop. about 15 min.



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