Erasmus+

INSTITUT PASTEUR

EDUCATION

Projects 2021-2022

Erasmus+

Institut Pasteur 2021-2022

RESEARCH CENTRE

Legal name: **Institut Pasteur**

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Brief description of your Institution

The Institut Pasteur is a private non-profit foundation that contributes to the prevention and treatment of diseases through research, education, and public health activities. Its campus in Paris hosts almost 2600 individuals.
Research: priority is given to fight infectious diseases, such as viral, bacterial, and parasitic diseases, as well as certain types of cancer, genetic, neurodegenerative, and allergic diseases.
Education: every year 550 young scientists from all over the world follow high-level courses in various fields related to research in microbiology, immunology, cellular biology, epidemiology, genetics, and disease control. Over 850 trainees from 60 different countries come to perfect their skills or conduct their Master or Doctoral trainings in the Institute's laboratories.

Description of the work program(s)

See projects on following pages

N° of placements available for work programs a), b), c) etc:

The laboratories at Pasteur have proposed 24 projects for Erasmus internships (see following pages). Students may also contact other laboratories at Pasteur to apply for an internship, even if the laboratories have not presented a project.

FACILITIES (not compulsory for the host centre)

- **Accommodation** (some centres offer it) **X** YES ☐ NO
  a limited number of rooms for rent are reserved for Pasteur at the student residence Cité Universitaire http://www.ciup.fr/
- **Support in finding accommodation** (many centres offer it) **X** YES ☐ NO
- **Canteen** (most centres offer it) **X** YES ☐ NO

- **Additional salary** (some centres offer an additional salary ranging from 200 to 1000 €/month) **X** YES ☐ NO
  additional salary of approximately 577 euros/month (depending on the number of working days) is paid by the host lab (3.90 euros/hour, 7 hours/ day)

Title of the work program 1

Role of the intermediate filament protein Synemin in mechanotransduction

Description of the work program

Glioblastoma multiforme (GBM), also known as astrocytoma grade IV, is the most common type of primary brain tumors with a very poor prognosis for patients. The invasive character of GBM is one of the main contributors to the poor prognosis as cells migrate away from the tumor core, evade therapy and initiate recurrence. Tumor invasion is also what ultimately causes the death of patients by altering essential brain tissues. Current diagnostic methods cannot identify the invasive cells and do not accurately predict tumor spreading. Hence, there is an urgent need for a molecular signature of GBM cell invasive properties. This requires fundamental knowledge on the biology of GBM and their mechanisms of invasion through the healthy brain parenchyma. One general goal of the lab projects is to identify molecular alterations characteristic of invading GBM cells.

Until now, the role of actin in cell migration has been extensively studied, but much less is known about the role of microtubules and Intermediate Filaments(IFs) (Dutour-Provenzano and Etienne-Manneville, 2021, 34033784; Etienne-Manneville, 2018, 30059630). Changes in IF composition are generally associated with tumor spreading, but the impact of IF composition on cell migratory behavior remains unknown. However several lines of evidence point to a role of IFs in cell migration (De Pascalis et al., 2018; Leduc and Etienne-Manneville, 2015, 25660489; Leduc and Etienne-Manneville, 2017, 28722513). The unique mechanical properties of IFs and their crosstalk with the other cytoskeletal networks hint at their participation in cell mechanics and mechanotransduction and therefore strongly suggest that IFs play an essential role in cell invasion in mechanically challenging microenvironments (van Bodegraven and Etienne-Manneville, 2021). Using in vitro models of astrocyte and glioblastoma cell migration we have demonstrated that (i) cell interaction with the extracellular matrix triggers signaling cascades leading to IF rearrangements (Leduc and Etienne-Manneville, 2017, 28432079), (ii) IFs control cell polarity and nucleus positioning (Dupin and Etienne-Manneville, 2011, 21959251; Dupin et al., 2011, 21378307) and (iii) IFs controls astrocyte migration by antagonizing the acto-myosin network (De Pascalis et al., 2018; van Bodegraven and Etienne-Manneville, 2020, 32623234). Preliminary observations from the lab point to Synemin as a key player of the IF network in astrocytes and glioblastoma cells. Synemin has been shown to interact with focal adhesions which makes it an excellent candidate to control mechanotransduction at these sites. Here we propose to manipulate IF protein expression in astrocytes and GBM cells to determine the fundamental mechanisms by which Synemin influence cell invasive behavior depending on the microenvironment.

The candidate will use recently generated Synemin-KO glioblastoma cell lines, and astrocytes transfected with siRNA targetting Synemin, to assess the role of this protein in the organization of IF network, the dynamics of focal adhesions, the generation and transmission of traction forces and in mechanosensitive migration (as done previously in Seetharaman et al. https://doi.org/10.1101/2020.07.22.205203)

The study will include

- Immunofluorescence and live cell imaging of the IF network and of the focal adhesions during cell migration
- A combination of biochemistry and traction force microscopy to determine the influence of Synemin on Rho/ROCK/myosin signaling and acto-myosin-mediated traction forces.
- Live cell imaging of cell migration on 2D substrates of various rigidities to determine the role of IFs in mechanosensitive cell migration.

This cell biology/mechanobiology project has a strong potential to generate new fundamental knowledge and key clinically relevant results. In particular, it will provide fundamental bases for the use of Synemin expression as diagnostic marker and possibly as therapeutic target to influence or prevent GBM cell invasion.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

De Pascalis, C., C. Pérez-González, S. Seetharaman, B. Boëda, B. Vianay, M. Burute, C. Leduc, N. Borghi, X. Trepat, and S. Etienne-Manneville. 2018. Intermediate filaments control collective migration by restricting traction forces and sustaining cell–cell contacts. *The Journal of Cell Biology*. 217:3031-3044.

Dupin, I., and S. Etienne-Manneville. 2011. Nuclear positioning: mechanisms and functions. *Int J Biochem Cell Biol*. 43:1698-1707.

Dupin, I., Y. Sakamoto, and S. Etienne-Manneville. 2011. Cytoplasmic intermediate filaments mediate actin-driven positioning of the nucleus. *J Cell Sci*. 124:865-872.

Dutour-Provenzano, G., and S. Etienne-Manneville. 2021. Intermediate filaments. *Curr Biol*. 31:R522-R529.

Etienne-Manneville, S. 2018. Cytoplasmic Intermediate Filaments in Cell Biology. *Annu Rev Cell Dev Biol*. 34:1-28.

Leduc, C., and S. Etienne-Manneville. 2015. Intermediate filaments in cell migration and invasion: the unusual suspects. *Curr Opin Cell Biol*. 32:102-112.

Leduc, C., and S. Etienne-Manneville. 2017. Intermediate filaments join the action. *Cell Cycle*. 16:1389-1390.

Leduc, C., and S. Etienne-Manneville. 2017. Regulation of microtubule-associated motors drives intermediate filament network polarization. *J Cell Biol*. 216:1689-1703.

van Bodegraven, E.J., and S. Etienne-Manneville. 2020. Intermediate filaments against actomyosin: the david and goliath of cell migration. *Curr Opin Cell Biol*. 66:79-88.

van Bodegraven, E.J., and S. Etienne-Manneville. 2021. Intermediate Filaments from Tissue Integrity to Single Molecule Mechanics. *Cells*. 10 1905.

Scientific or technical background required for work program

We are looking for highly motivated student with a strong interest in cell biology and cancer biology. Ideally, the candidate would have a strong scientific background in cell biology or in biophysics. Previous lab experience in cell biology, live cell imaging, mechanobiology or image analysis would be a plus.

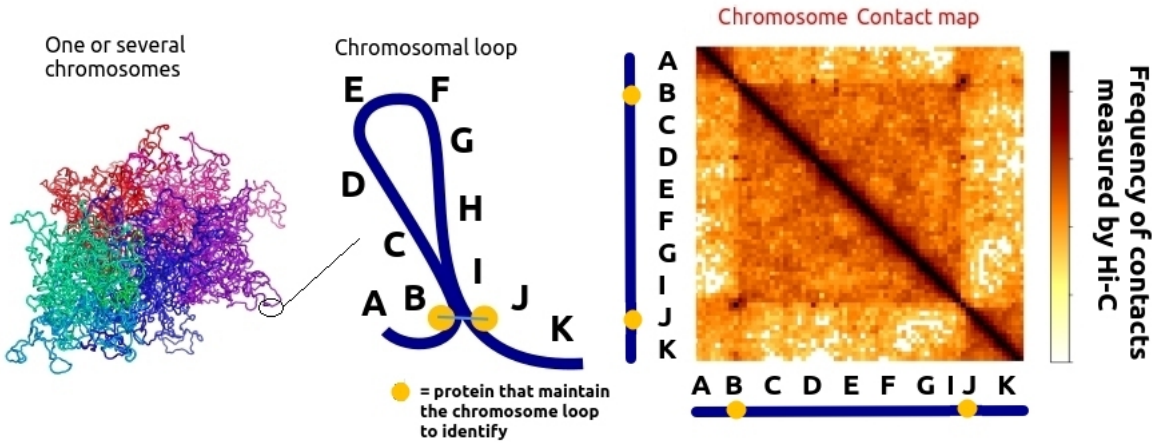
Title of the work program 2

Long range chromosome loops in the budding yeast

Description of the work program

It is becoming increasingly clear that **chromosome architecture** is optimized for the proper execution of biological functions such as gene expression and replication, and that these in turn constrain the spatial organization of genomes on certain scales. Our community has recently identified several key proteins behind chromosome architecture and several associated mechanisms. The spatial organization of genomes can change during a cancer process (Taberlay et al. Genome Research 2016) or during a viral infection (Heinz et al., Cell 2018). A detailed knowledge of the mechanisms underlying chromosome architecture is thus important for a better understanding of certain pathologies.

The yeast *Saccharomyces cerevisiae* is a prime model organism. It is a eukaryotic cell that has the advantages of microorganisms (ease of cultivation, genetic manipulation) and several points in common with a human cell (rolling of DNA into nucleosomes, epigenetic etc). To observe the 3D structure of chromosomes, we use a technology called **Hi-C** (Lieberman-Aiden et al. Science 2009). This technique is based on the capture and sequencing at high throughput of DNA fragments that are close to each other (Dekker et al. 2002, Figure). This makes it possible to measure the contact frequencies between different loci within a chromosome or between two chromosomes and thus to infer the 3D organisation of genome. Our laboratory has recently identified stable chromosome loops of several tens of kilobases (kb) in yeast chromosomes during certain moments of the cell cycle (in mitosis: Garcia-Luis et al. NSMB 2019, Dauban et al. Mol. Cell 2020, in meiosis: Muller et al. MSB 2018).



We recently observed the presence of long range loops (> 50 kb) in different conditions that are independent of cohesin (unpublished data). The objective of this project is to **identify the biological and physical factors behind the formation and maintenance of this new type of loops.** These long-range loops could be linked to transcription activity and formed by collective processes like those of a phase transition.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Computer vision for pattern detection in chromosome contact maps.
Matthey-Doret C, Baudry L, Breuer A, Montagne R, Guiglielmoni N, Scolari V, Jean E, Campeas A, Chanut PH, Oriol E, Méot A, Politis L, Vigouroux A, Moreau P, Koszul R#, **Cournac A.#** Nature Commun. 2020 Nov 16;11(1):5795. doi: 10.1038/s41467-020-19562-7. PMID: 33199682

Tridimensional infiltration of DNA viruses into the host genome shows preferential contact with active chromatin.
Moreau P*, **Cournac A***, Palumbo GA, Marbouty M, Mortaza S, Thierry A, Cairo S, Lavigne M, Koszul R, Neuveut C. Nat Commun. 2018 Oct 15;9(1):4268. doi: 10.1038/s41467-018-06739-4. PMID: 30323189

Multiscale Structuring of the E. coli Chromosome by Nucleoid-Associated and Condensin Proteins.
Lioy VS*, **Cournac A***, Marbouty M, Duigou S, Mozziconacci J, Espéli O, Boccard F, Koszul R. Cell. 2018 Feb 8;172(4):771-783.e18. doi: 10.1016/j.cell.2017.12.027. Epub 2018 Jan 18. PMID: 29358050

(* indicate co-first authors, # indicate corresponding authors)

Scientific or technical background required for work program

The project we propose is an interdisciplinary project at the interface between *molecular biology* and *bioinformatics* analysis.

Techniques used: experimental classical molecular biology techniques (culture, PCR, Hi-C etc), computational (pipelines developed within the team in python language, user friendly). Depending on the abilities of the candidate, the student will have the choice to devote more time to the experimental or computer part of the project with training given in the laboratory.

Title of the work program 3

Choroid plexus activity and its role in shaping brain function, in health and aging

Description of the work program

Aging-associated dementia and neurodegenerative diseases, such as Alzheimer's disease, have become a major societal problem, but despite considerable efforts and resources devoted to developing therapies to combat them, we are still missing a cure. This last decade has seen the emergence of the concept that brain function is shaped and influenced by various biological factors remote from the brain, such as gut microbiota and systemic immune system. While the brain was considered to be isolated from systemic circulation by strong barriers, studies have identified communication patterns and circuits between the gut, blood and brain, but these remain poorly understood.

Intriguingly, one of the barriers between blood and brain, the choroid plexus, shows strong immunological activity and variation with aging. The choroid plexus is a single epithelial layer that separates the fenestrated blood vessels from the cerebrospinal fluid in the brain ventricles. This unique structure makes this tissue an interesting site for immune-brain cross-talks.

Our lab aims to explore activity at the choroid plexuses in health and in aging and determine how it shapes brain function. Based on results of comparative transcriptomic analyses performed on choroid plexuses single cells, we propose to genetically modify the expression of the genes of interest in the choroid plexuses in a mouse model, using CRISPR/Cas9 technology and study the associated phenotype combining immunofluorescence, flow cytometry and mice behavioral testing approaches.

The Erasmus student will participate at all the stages of the development and the realization of this project. The student will be particularly involved in molecular biology steps required for cloning targets of the genes of interest, in cellular biology approaches necessary for viral production of particles containing the genetic targets and that will be injected in choroid plexuses and in the analysis of the phenotype induced by the genetic modification of the choroid plexuses.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Deczkowska A*, Keren-Shaul H*, Weiner A*, Colonna M, Schwartz M, Amit I, Disease-associated microglia: a universal immune sensor of neurodegeneration, *Cell*, 2018.

Schwartz M and **Deczkowska A**, Neurological Disease as a Failure of Brain-Immune Crosstalk: The multiple faces of neuroinflammation, *Trends in immunology* 2016.

Baruch K*, **Deczkowska A***, David E, Castellano JM, Miller O, Kertser A, Berkutzki T, Barnett-Itzhaki Z, Bezalel D, Wyss-Coray T, Amit I, Schwartz M. Aging-induced type I interferon response at the choroid plexus negatively affects brain function, *Science* 2014 *equal authors.

Baruch K, **Deczkowska A**, Rosenzweig N, Tsitsou-Kampeli A, Sharif AM, Matcovitch-Natan O, Kertser A, David E, Amit I, Schwartz M., PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease, *Nat.Med.* 2016.

Deczkowska A*, Matcovitch-Natan O*, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, Singer O, David E, Winter DR, Smith LK, Kertser A, Baruch K, Rosenzweig N, Terem A, Prinz M, Villeda S, Citri A, Amit I, Schwartz M. Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. Nat.Comm. 2017 *equal authors.

Scientific or technical background required for work program

- Lab experience, molecular biology and cellular biology experience.
- Must be OK with animal experimentation.
- Strong work ethics.
- Scientific curiosity.
- Ability to integrate in a new work environment and work in a team.

Title of the work program 4

Deciphering the impact of telomerase on antigenic variation in African trypanosomes

Description of the work program

Trypanosoma brucei (T. brucei) causes both Human African and Animal African Trypanosomiasis and is fatal if left untreated. Trypanosomes are transmitted by the bite of the tsetse fly to the mammalian host where they exist in the bloodstream and tissue. Parasite survival relies on the ability to evade the host's immune system by antigenic variation, or the stochastic switching of the variant surface glycoprotein (VSG) and is essential for the long-term survival in the mammalian host. VSG switching occurs most commonly by homologous recombination (HR), and several DNA repair proteins are important for this process. However, very little is understood on how the cells accesses a vast subtelomeric repertoire of VSG genes or how a recombination-based switch is resolved to result in an intact bloodstream form expression site (BES). We propose that telomerase reverse transcriptase (TERT) is required to resolve a VSG switch and modulated access to the VSG repertoire. In this project we will generating TERT nulls in our Trypanosomes strains that allow use to induced single DNA breaks at a BES and investigate VSG switching using VSG-seq or high throughput sequencing of switched populations.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. **Glover L**, Jun J, Horn D. Microhomology-mediated deletion and gene conversion in African trypanosomes. Nucleic Acids Res. 2011;39(4):1372-80.
2. **Glover L**, Alsford S, Horn D. DNA break site at fragile subtelomeres determines probability and mechanism of antigenic variation in African trypanosomes. PLoS Pathog. 2013;9(3):e1003260.
3. Rudd SG, **Glover L**, Jozwiakowski SK, Horn D, Doherty AJ. PPL2 translesion polymerase is essential for the completion of chromosomal DNA replication in the African trypanosome. Mol Cell. 2013;52(4):554-65.
4. **Glover L**, Horn D. Locus-specific control of DNA resection and suppression of subtelomeric VSG recombination by HAT3 in the African trypanosome. Nucleic Acids Res. 2014;42(20):12600-13.
5. **Glover L**, Alsford S, Baker N, Turner DJ, Sanchez-Flores A, Hutchinson S, Hertz-Fowler C, Berriman M, Horn D. Genome-scale RNAi screens for high-throughput phenotyping in bloodstream-form African trypanosomes. nature protocols. 2015;10(1):106-33. Epub 2014/12/17. doi: 10.1038/nprot.2015.005. PubMed PMID: 25502887.
6. **Glover L**, Hutchinson S, Alsford S, Horn D. VEX1 controls the allelic exclusion required for antigenic variation in trypanosomes. Proceedings of the National Academy of Sciences. 2016;113(26):7225-30.

7. Rico E, Ivens A, **Glover L**, Horn D, Matthews KR. Genome-wide RNAi selection identifies a regulator of transmission stage-enriched gene families and cell-type differentiation in Trypanosoma brucei. PLoS Pathog. 2017;13(3):e1006279.
8. Faria J, **Glover L**, Hutchinson S, Boehm C, Field MC, Horn D. Monoallelic expression and epigenetic inheritance sustained by a Trypanosoma brucei variant surface glycoprotein exclusion complex. Nature communications. 2019;10(1):1-14. Epub 2019/07/11. doi: 10.1038/s41467-019-10823-8. PubMed PMID: 31289266; PMCID: PMC6617441. pasteur-03107015**, version 1**
9. **Glover L***, Marques CA, Suska O, Horn D*. Persistent DNA damage foci and DNA replication with a broken chromosome in the African trypanosome. MBio. 2019;10(4):e01252-19. pasteur-03107118**, version 1**
10. Mehnert AK, Prorocic M, Dujeancourt-Henry A, Hutchinson S, McCulloch R, **Glover L**. The MRN complex promotes DNA repair by homologous recombination and restrains antigenic variation in African trypanosomes. Nucleic Acids Res. 2021. Epub 2021/01/16. doi: 10.1093/nar/gkaa1265. PubMed PMID: 33450001. pasteur-03107143

Scientific or technical background required for work program

We are looking for a student who is enthusiastic and has some experience in molecular biology. Experience with Next Generation sequencing-based techniques, computational analysis, and cell culture is a great advantage, but prior experience in parasitology is not required.

They must have good scientific writing and communication skills in English

Title of the work program 5

The effect of existing and newly developed antibiotics on the gut microbiota.

Description of the work program

The proposed project is part of the NASPEC research project: Narrow spectrum antibiotics to fight the emergence of bacterial resistance. The three objectives of NASPEC are as follows (i) identifying strategies to alleviate the drug selective pressure on the intestinal flora and limiting the emergence of resistance, (ii) developing drugs that are selectively active on the bacteria listed as critical priorities by the WHO, *i.e.,* carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, carbapenem-resistant and 3rd generation cephalosporin-resistant Enterobacteriaceae, and (iii) determining how drugs specifically targeting MDR pathogens could be developed and used to minimize microbiota dysbiosis and deleterious host immune responses.

Our team is responsible for high-throughput sequencing and bioinformatic analysis of samples from humanized mouse models treated with different antibiotic compounds. The successful candidate will aid in data analysis whose goal is to provide information concerning the efficacy of various existing and newly developed compounds. This includes information on drugs to selectively target specific pathogens while preserving commensal bacterial species in the intestinal flora.

The selected candidate will participate in group discussions and the presentation of results and reports for both the laboratory team as well as for the larger project. They will gain a deeper understanding of sequencing technology, computational analysis and python programming.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. Volant, S. *et al.* SHAMAN: a user-friendly website for metatagenomic analysis from raw reads to statistical analysis. *BMC Bioinformatics* **21**, 345 (2020).
2. Ruppe, E. *et al.* Prediction of the intestinal resistome by a three-dimensional structure-based method. *Nature microbiology* **4**, 112-+ (2019).
3. Lanza, V. F. *et al.* In-depth resistome analysis by targeted metagenomics. *Microbiome* **6**, 11 (2018).
4. de Gunzburg, J. *et al.* Protection of the Human Gut Microbiome From Antibiotics. *J Infect Dis* **217**, 628–636 (2018).
5. Quereda, J. J. *et al.* Bacteriocin from epidemic Listeria strains alters the host intestinal microbiota to favor infection. *Proc Natl Acad Sci U S A* **113**, 5706–11 (2016).

6. Chatelier, E. L. *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).

Scientific or technical background required for work program

- Basic knowledge in programming or strong desire to learn programming and Python.
- Strong interest in microbiology and metagenomics/microbiota.

Title of the work program 6

Targeted optogenetic manipulation of auditory learning

Description of the work program

Driving perception by direct activation of neural ensembles in cortex is a necessary step for achieving a causal understanding of the neural code for auditory perception and developing central sensory rehabilitation methods. The higher brain structure of the auditory system is the auditory cortex.

In this project, we will use patterned activation of the auditory cortex in mice coupled with a behavioural task to investigate the features of the auditory code that are facilitating discriminative learning of sounds. Activation will be obtained with the optogenetic technology that allow activating genetically identified neurons by light. In particular, we will dissect the role of neuronal activity time course in learning. This project will involve animal behaviour, optogenetics, electrophysiology and data analysis.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Schwenkgrub J., Harrell ER, Bathellier B.*, Bouvier J.*, Deep imaging in the brainstem reveals functional heterogeneity in V2a neurons controlling locomotion, Science Advances, 2020, 6:eabc6309

Harrell ER., Goldin MA., Bathellier B., Shulz DE., An elaborate sweep-stick code in rat barrel cortex, Science Advances, 2020, 6:eabb7189

Ceballo S., Piwkowska Z., Bourg J., Daret A., Bathellier B., Targeted Cortical Manipulation of Auditory Perception, Neuron, 2019,104:1168-1179

Scientific or technical background required for work program

Biology, Physics, Engineering, Neuroscience background is an asset

Title of the work program 7

Host metabolism and malaria infection

Description of the work program

Malaria remains a major cause of death and morbidity worldwide, and yet there is no cure nor effective vaccine. Malaria infection begins in the liver with invasion of hepatocytes by *Plasmodium* parasites. Despite clear parasitism and subversion of host metabolic networks, the molecular mechanisms implicated in parasite growth and replication inside hepatocytes remain largely unknown. The work program proposed here will involve the characterisation of novel regulators of malaria infection in hepatic cells using genetics, biochemistry, advanced live-cell microscopy, and high-content imaging.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Vera IM, Grilo Ruivo MT, Lemos Rocha LF, Marques S, Bhatia SN, Mota MM, Mancio-Silva L. Targeting liver stage malaria with metformin. JCI Insight. 2019 Dec 19;4(24):e127441. doi: 10.1172/jci.insight.127441. PMID: 31852843

Mancio-Silva L, Fleming HE, Miller AB, Milstein S, Liebow A, Haslett P, Sepp-Lorenzino L, Bhatia SN. Improving Drug Discovery by Nucleic Acid Delivery in Engineered Human Microlivers. *Cell Metabolism*. 2019 Mar 5;29(3):727-735.e3. doi: 10.1016/j.cmet.2019.02.003. PMID: 30840913.

Gural N, Mancio-Silva L, He J, Bhatia SN. Engineered Livers for Infectious Diseases. Cell Mol Gastroenterol Hepatol. 2017 Nov 22;5(2):131-144. doi: 10.1016/j.jcmgh.2017.11.005. eCollection 2018. PMID: 29322086

Mancio-Silva L, Slavic K, Grilo Ruivo MT, Grosso AR, Modrzynska KK, Vera IM, Sales-Dias J, Gomes AR, MacPherson CR, Crozet P, Adamo M, Baena-Gonzalez E, Tewari R, Llinás M, Billker O, Mota MM. Nutrient sensing modulates malaria parasite virulence. *Nature*. 2017 Jul 13;547(7662):213-216. doi: 10.1038/nature23009. Epub 2017 Jul 5. PMID: 28678779.

Ruivo MTG, Vera IM, Sales-Dias J, Meireles P, Gural N, Bhatia SN, Mota MM, Mancio-Silva L. Host AMPK Is a Modulator of Plasmodium Liver Infection. *Cell Reports.* 2016 Sep 6;16(10):2539-2545. doi: 10.1016/j.celrep.2016.08.001. Epub 2016 Aug 25. PMID: 27568570.

Scientific or technical background required for work program

Applicants should have interest in addressing cell biological or biochemical problems, be highly motivated and enjoy working in an interactive, collaborative, and international environment.

Title of the work program 8

Molecular bases for ATG16L1 interactions with multiple proteins

Description of the work program

ATG16L1 was identified in higher eukaryotes for its resemblance to Atg16, a yeast protein that plays a key role in autophagy. ATG16L1 has a carboxy-terminal WD40 domain (WDD), absent in Atg16, that enabled the diversification of its functions. Several proteins have been shown to bind the WDD, with important consequences on their activities, for instance in the signalling by the receptors to the cytokines IL-2 and IL-10. However, the interaction surface(s) on the WDD have not been mapped for any of the WDD interactors.

The host lab has discovered a bacterial protein, TaiP, that binds to WDD. In collaboration with the Schreiber laboratory (Weizmann Institute, Israel), a yeast display strategy was implemented to identify residues in WDD, that are implicated in TaiP/WDD interaction. The intern will test these predictions by expressing mutated forms of the WDD in human cells and test their ability to enhance or decrease TaiP/WDD interaction. The loss-of-functions mutants will then be tested in functional assays involving other WDD binders (i) traffic and signalling by the cytokine receptors IL-2Rγ and IL-10RB, (ii) NOD2-dependent signalling during *Shigella* infection. Complementary to these functional assays, pull-down and co-immunoprecipitation experiments will determine whether all WDD binders compete for the same interaction surfaces or not.

The intern will utilize a wide variety of techniques in cell culture (transfections, gene silencing), molecular biology (plasmid design), biochemistry (co-immunoprecipitation, pull-down) and microscopy (immunofluorescence, videomicroscopy).

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. Hamaoui, D, Cossé, M.M., Mohan, J. Lystad, A.H., Wollert, T. and Subtil, A. The *Chlamydia* effector CT622/TaiP targets a non-autophagy related function of ATG16L1 (2020) **PNAS** 117, 26784-26794 doi:10.1073/pnas.2005389117
2. Hamaoui, D., and Subtil, A. (2021). ATG16L1 functions in cell homeostasis beyond autophagy. **FEBS J**. doi 10.1111/febs.15833
3. Maffei, B., Laverriere, M., Wu, Y., Triboulet, S., Perrinet, S., Duchateau, M., Matondo, M., Hollis, R. L., Gourley, C., Rupp, J., Keillor, J. W., and Subtil A Infection-driven activation of transglutaminase 2 boosts glucose uptake and hexosamine biosynthesis in epithelial cells(2020). **EMBO J.** 39, e102166 doi 10.15252/embj.2019102166
4. Cossé M.M., Barta ML, Fiser DJ, Oesterlin LK, Niragire B, Perrinet S, Millot GA, Hefty PS, Subtil A The loss of expression of a single type 3 effector (CT622) strongly reduces *Chlamydia trachomatis* infectivity and growth (2018) **Front Cell Infect Microbiol**. 8:145 doi 10.3389/fcimb.2018.00145

Scientific or technical background required for work program

A good background in cell biology and biochemistry is recommended. Previous experience at the bench will be appreciated.

Title of the work program 9

Epigenetic control of cell fate decisions in development and cancer

Description of the work program

How the identity of mature cells is maintained in multicellular organisms and how it is subverted in disease, particularly in cancer, are essential questions in biology and medicine. Cellular identity is dictated by transcriptional programs mediated by specific chromatin configurations, yet the mechanisms by which cell fate decisions are choreographed to generate the adult organism or are corrupted in tumors remain poorly understood. Notably, the role of post-translational modifications of proteins by other proteins in this process is still enigmatic. Among these, modification of chromatin proteins by the small SUMO protein acts as a general mechanism that safeguards cellular identity. Lowering the SUMO barriers facilitates change in cell identity irrespective of type and is able to generate embryo-like structures from embryonic stem cells.

The main objective of this project is to decipher the **chromatin role of SUMO in cell plasticity**, with a particular focus on embryonic and adult stem cells, with the ultimate goal of exploiting this knowledge to explore **cancer cell reprogramming and in vitro embryo development modeling**. The project will involve single cell -omics, chromatin biology, genome editing, protein biochemistry, live imaging as well as embryoid and organoid model systems.

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Selected publications or patents of the Research Group offering the work program

Theurillat I*, Hendriks I*, Cossec J-C*, Andrieux A, Nielsen M and Dejean A. Extensive SUMO modification of repressive chromatin factors distinguishes pluripotent from somatic cells. (*first authors) ***Cell Rep,*** 2020, 32 (11): 1081 46.

Cossec J-C*, Theurillat I*, Chica C, Bua Aguin S, Gaume X, Andrieux A, Iturbide A, Jouvion G, Li H, Bossis G, Seeler J-S, Torres-Padilla ME and A Dejean. SUMO safeguards somatic and pluripotent cell identities by enforcing distinct chromatin states. (*first authors) ***Cell Stem Cell,*** 2018, 23(5):742-757.

Seeler JS and Dejean A. SUMO and the robustness of cancer. ***Nat Rev Cancer***, 2017,17(3):184-197.

Decque A, Joffre O, Magalhaes J.G, Cossec J-C, Blecher-Gonen R, Seeler J-S, Lapaquette P, Silvin A, Joubert P-E, Albert M.L, Amit I, Amigorena S and Dejean A. Sumoylation coordinates the repression of inflammatory and anti-viral gene programs during innate sensing. ***Nat Immunol***, 2016, 17, 140-149.

Scientific or technical background required for work program

Candidates should have interest in epigenetics and stem cells. Prior experience in tumor models, embryology and/or protein biochemistry are not mandatory but will be a plus.

Title of the work program 10

Impact of senescence on cellular reprogramming

Description of the work program

In this project, we aim to continue investigate the interplay between senescence and cellular reprogramming. We will use a wide range of approaches, combining senescence induction, single cell transcriptomic, in vitro reprogramming and novel in vivo reprogrammable mouse models to investigate whether/how reprogramming process might impact senescence program.

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Selected publications or patents of the Research Group offering the work program

1. von Joest M, Chen C, Douché T, Chiche A, Gianetto QG, Matondo M, **Li H**. 2021. Amphiregulin mediates non-cell-autonomous effect of senescence on reprogramming. *bioRxiv*. doi:10.1101/2021.09.01.458621

2. Chiche A, Le Roux I, von Joest M, Sakai H, Aguín SB, Cazin C, Salam R, Fiette L, Alegria O, Flamant P, Tajbakhsh S, **Li H**. 2017. Injury-Induced Senescence Enables In Vivo Reprogramming in Skeletal Muscle. *Cell Stem Cell.* **20**.

3. **Li H**, Collado M, Villasante A, Matheu A, Lynch CJ, Cañamero M, Rizzoti K, Carneiro C, Martinez G, Vidal A, Lovell-Badge R, Serrano M. 2012. p27Kip1 directly contributes to Sox2 transcriptional repression during embryonic stem cell differentiation. *Cell Stem Cell*. **11.**

4. **Li H**, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M. 2009. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature*. **460**.

Scientific or technical background required for work program

Senescence elimination and partial reprogramming, induced by short-term OSKM expression, are considered emerging strategies for rejuvenation[2]. One exciting perspective is combining them for more effective rejuvenation and less potential detrimental effects. Interestingly, senescence and reprogramming are intimated linked. In particular, senescence has both cell- and non-cell-autonomous effects on reprogramming. Although it is well established that proliferation arrest is a cell-intrinsic barrier for reprogramming, the mechanisms by which senescence regulates cell plasticity to promote cell fate conversion are not well understood. Moreover, the role of reprogramming in modulating senescence program is mostly unknown. We aim to understand the crosstalk between senescence and reprogramming in the context of cancer and aging. In this program, we will leverage on systems well established in the lab to explore whether and how reprogramming factors could modulate senescence program, and its potential implication in aging-associated diseases.

Title of the work program 11

Spatially resolved proteomics of GPCR-coupled RhoGEFs controlling the emergence of haematopoietic stem cells.

Description of the work program

The work program will fully integrate into one of our main interests in the laboratory which is to decipher the cellular and molecular principles underlying the emergence of hematopoietic stem cells from aortic vessels.
For this purpose, we are currently developing a challenging approach of proximity biotinylation using proteins of interest fused with the enzyme ascorbate peroxidase as bait (APEX2 technology). These proteins (Rho Exchange Factors or GEFs) are involved in the coupling between G-protein Coupled Receptors (GPCRs) activity and Rho-GTPases regulating the structure/dynamics of actin, in particular at the junctional interface between aortic cells and emerging hematopoietic precursor cells. This approach is challenging because not yet routinely applied to *in vivo* model systems. In our case, we use the zebrafish embryo as a model which is very powerful to investigate processes conserved throughout vertebrate species (and so is the emergence, during development, of hematopoietic stem cells).
The student will learn the handling of house-made transgenic zebrafish lines for crosses and obtaining embryos; she/he will contribute optimizing (i) conditions for biotinylation *in vivo*, (ii) isolation of biotinylated protein complexes to be analysed quantitatively by mass spectrometry. The work will be performed in collaboration with engineers of the proteomic platform of the Pasteur Institute. *In vivo* imaging using confocal microscopy will also be performed so as to control physiological parameters and hematopoiesis efficiency. Depending at which time the student will arrive (or how long she/he will stay), live microscopy will also be used to investigate phenotypes triggered by interfering with the expression of proteins that will be identified as partners of the GPCR-coupled RhoGEFs used as bait.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Lancino M, Majello S, Herbert S, De Chaumont F, Tinevez J-Y, Olivo-Marin J-C, Herbomel P, Schmidt A. 2018. Anisotropic organization of circumferential actomyosin characterizes hematopoietic stem cells emergence in the zebrafish. Elife 7. doi:10.7554/eLife.37355

Renard H-F, Simunovic M, Lemière J, Boucrot E, Garcia-Castillo MD, Arumugam S, Chambon V, Lamaze C, Wunder C, Kenworthy AK, Schmidt AA, McMahon HT, Sykes C, Bassereau P, Johannes L. 2015. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. Nature 517:493–496. doi:10.1038/nature14064

Vannier C, Pesty A, San-Roman MJ, Schmidt AA. 2013. The Bin/amphiphysin/Rvs (BAR) domain protein endophilin B2 interacts with plectin and controls perinuclear cytoskeletal architecture. J Biol Chem 288:27619–27637. doi:10.1074/jbc.M113.485482

Scientific or technical background required for work program

The student is expected to have acquired knowledge in biochemical approaches (protein biochemistry, affinity chromatography, protein analyses (ex: SDS/PAGE, blotting)) as well as, possibly (but it is not mandatory), confocal microscopy. Having an interest in *in vivo* animal models would be an advantage (in particular having acquired a small experience with the zebrafish) but there is no obligation. He/she should have strong organization skills and should be strongly motivated by bench work.

Title of the work program 12

Role of human auto-antibodies to nicotinic receptors in neurological and psychiatric disease

Description of the work program

Mental disorders are the most debilitating and costly diseases in developed countries. It is estimated that 38.2% of the European population is affected at some point in their lives (*1*). For many diseases, no adequate treatments are available. There is a growing realisation that the immune system may be a key player. For example, in a major genome-wide association study (*GWAS*) linking genetic alterations to schizophrenia, the Major Histocompatibility Complex (MHC) locus was the major "hit" in the genome (*2*), far ahead of any genes expressed in the central nervous system.

Psychotic disorders, encompassing **schizophrenia (SZ)** and **bipolar disorders (BD)**, are major health problems worldwide. Due to their complexity, their heterogeneity and the absence of biomarkers to identify homogeneous subgroups, our understanding of the causes of psychotic disorders remains limited and hence, development of new pathway-related treatment is hampered. However, it is now well-established that immune dysfunction, including auto-immunity, are clearly associated with psychotic disorders opening up new avenues for the discovery of biomarkers, the understanding of mechanisms and the implementation of innovative treatments. Furthermore, the importance of auto-immunity in major psychosis has been strongly reinforced by the association of auto-immune disorders with psychotic disorders (*3*) and by the discovery of anti-neuronal auto-antibodies (AAbs) that alter synaptic transmission, such as the anti-NMDA receptor antibodies (NMDAR-AAb) in what is called “auto-immune psychosis” (*4*) (*5*). Thus, the role of auto-immunity against neuronal receptor targets in the pathogenesis of psychotic disorders has gained tremendous support (*6*) over the last years and urgently requires multidisciplinary in-depth investigations to explore new AAbs against brain receptors, to characterise patients carrying these AAbs and to unravel the molecular mechanisms underlying psychosis in order to offer new therapeutic strategies in homogeneous subgroups of patients.

Here, we plan to tackle such a major challenge in a unique, complementary and synergistic consortium of clinical and basic research experts, with Harald Prüß (MD, Germany), and Marion Leboyer and Ryad Tamouza (MDs, France).

These partners have already strongly contributed to our current project in improving the understanding of auto-immunity in neuropsychiatric disorders. Given the preliminary data obtained with our collaborators, we propose here a comprehensive analysis, and dissection, of the ***origin***, ***role*** and ***consequences*** **of AAbs against nicotinic acetylcholine receptor (nAChR) proteins**, discovered by the Maskos group as ***novel biomarkers***, in patients and rodent models. In addition, the genetics of the ***MHC/HLA*** complex of the patients will be analysed further given that Genome-wide association studies (GWAS) of the “archetypical” nAChR auto-immune disease, *myasthenia gravis*, have identified first links to HLA (*7*).

References

1. H. U. Wittchen, et al., The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur. Neuropsychopharmacol.* **21**, 655–679 (2011).
2. S. Ripke, et al., Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. **511**, 421–427 (2014).
3. O. Köhler-Forsberg, P. B. Mortensen, L. Petersen, O. Mors, C. Gasse, S. Dalsgaard, R. H. Yolken, M. E. Benros, A Nationwide Study in Denmark of the Association Between Treated Infections and the Subsequent Risk of Treated Mental Disorders in Children and Adolescents. *JAMA Psychiatry*. **76**, 271–279 (2018).
4. T. A. Pollak, et al., Autoimmune psychosis: an international consensus on an approach to the diagnosis and

management of psychosis of suspected autoimmune origin. *The Lancet Psychiatry*. **7**, 93–108 (2020).
5. P. Ellul, L. Groc, R. Tamouza, M. Leboyer, The clinical challenge of autoimmune psychosis: Learning from anti-NMDA receptor autoantibodies. *Front. Psychiatry*. **8**, 1–6 (2017).
6. J. Jézéquel, V. Rogemond, T. Pollak, M. Lepleux, L. Jacobson, H. Gréa, C. Iyegbe, R. Kahn, P. McGuire, A. Vincent, J. Honnorat, M. Leboyer, L. Groc, Cell- and Single Molecule-Based Methods to Detect Anti-N-Methyl-D-Aspartate Receptor Autoantibodies in Patients With First-Episode Psychosis From the OPTiMiSE Project. *Biol. Psychiatry*. **82**, 766–772 (2017).
7. P. K. Gregersen, et al. Risk for myasthenia gravis maps to a 151Pro→Ala change in TNIP1 and to human leukocyte antigen-B*08. *Ann. Neurol.* **72**, 927–935 (2012).

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

S Lombardo, J Catteau, M Besson & U Maskos (2016) A role for ß2* nicotinic receptors in a model of local amyloid pathology induced in dentate gyrus. *Neurobiology of Aging* 46, 221-234.

F Koukouli, M Rooy, JP Changeux & U Maskos (2016) Nicotinic receptors in mouse prefrontal cortex modulate ultraslow fluctuations related to conscious processing. *Proc Natl Acad Sci U S A* 113, 14823-14828.

F Koukouli, M Rooy & U Maskos (2016) Early and progressive deficit of neuronal activity patterns in a model of local amyloid pathology in mouse prefrontal cortex. *Aging* 8, 3430-3449.

C Deflorio, S Blanchard, MC Carisì, D Bohl & U Maskos (2017) Human polymorphisms in nicotinic receptors: a functional analysis in iPS derived dopaminergic neurons. *FASEB J* 31, 828-839

F Koukouli, M Rooy, D Tziotis, KA Sailor, HC O'Neill, J Levenga, M Witte, M Nilges, JP Changeux, CA Hoeffer, JA Stitzel, BS Gutkin, DA DiGregorio & U Maskos (2017) Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. *Nature Medicine* 23, 347-354.

B Forget, P Scholze, F Langa, C Morel, S Pons, S Mondoloni, M Besson, R Durand-de Cuttoli, A Hay, L Tricoire, B Lambolez, A Mourot, P Faure & U Maskos (2018) A human polymorphism in *CHRNA5* is linked to relapse to nicotine seeking in transgenic rats. *Curr Biol* 28, 3244-3253.

R D'Alessio, F Koukouli, S Blanchard, J Catteau, C Rais, T Lemonnier, O Féraud, A Bennaceur-Griscelli, M Groszer & U Maskos (2020) Long-term development of human iPSC-derived pyramidal neurons quantified after transplantation into the neonatal mouse cortex. *Dev Biol* 461, 86-95

A Vitrac, S Pons, C Rais, M Balkota, N Lemière, J-P Bourgeois, U Maskos, T Bourgeron & I Cloëz-Tayarani (2020) A chimeric mouse model to study *in vivo* human iPSC-derived neurons: the case of a truncating SHANK3 mutation. *Sci Rep* 10(1):13315. doi: 10.1038/s41598-020-70056-4.

B Forget, R Icick, J Robert, C Correia, MS Prevost, M Gielen, P-J Corringer, F Bellivier, F Vorspan, M Besson & U Maskos (2020) Alterations in nicotinic receptor alpha5 subunit gene differentially impact early and later stages of cocaine addiction: a translational study in transgenic rats and patients. *Prog Neurobiol* 101898.doi: 10.1016/j.pneurobio.2020.101898.

Scientific or technical background required for work program

The candidate should have experience in basic molecular biology techniques, cell culture, ELISA. For highly motivated candidates, this training can also be provided as part of the internship, that should then be longer than six months.

Title of the work program 13

Characterization of HIV-1 and SARS-CoV-2 receptors

Description of the work program

Our group focuses on the dissection of the viral entry process with the aim to identify new therapeutic targets. The entry of HIV-1 and SARS-CoV-2 requires the interaction of a viral glycoprotein (gp120/Spike) with cellular receptors: CD4 and CCR5 for HIV-1, ACE2 for SARS-CoV-2. The objective of the intership will be to characterize the mechanisms that regulate the cell surface expression of these receptors by studying their organization at the plasma membrane (distribution, stoichiometry, dynamics) depending on different parameters (ligands, partners, membrane composition). For this, molecular biology, cell biology, and imaging approaches will be developed.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

- Gaelle Boncompain, Floriane Herit, Sarah Tessier, Aurianne Lescure, Elaine Del Nery, Pierre Gestraud, Isabelle Staropoli, Yuko Fukata, Masaki Fukata, Anne Brelot, Florence Niedergang, and Franck Perez. (2019). Targeting CCR5 trafficking to inhibit HIV-1 infection. Science Advances, Oct 16;5(10).
- Colin P, Zhou Z, Staropoli I, Garcia-Perez J, Gasser R, Armani-Tourret M, Benureau Y, Gonzalez N, Jin J, Connell BJ, Raymond S, Delobel P, Izopet J, Lortat-Jacob H, Alcami J, Arenzana-Seisdedos F, Brelot A, Lagane B. (2018). CCR5 structural plasticity shapes HIV-1 phenotypic properties. PLoS Pathog. 2018 Dec 6;14(12):e1007432.
- Brelot A, Chakrabarti LA (2018). CCR5 revisited: How mechanisms of HIV Entry govern AIDS pathogenesis J Mol Biol. 2018 Aug 17;430(17):2557-2589.
- Jin J, Momboisse F, Boncompain G, Koensgen F, Zhou Z, Cordeiro N, Arenzana-Seisdedos F, Perez F, Lagane B, Kellenberger E, Brelot A. (2018). CCR5 adopts three homodimeric conformations that control cell surface delivery. Science Signaling May 8;11(529).
- Jin J, Colin P, Staropoli I, Lima-Fernandes E, Ferret C, Demir A, Rogée S, Hartley O, Randriamampita C, Scott MG, Marullo S, Sauvonnet N, Arenzana-Seisdedos F, Lagane B, Brelot A. (2014). Targeting spare CC chemokine receptor 5 (CCR5) as a principle to inhibit HIV-1 entry. J Biol Chem. Jul 4;289(27):19042-52.
- P. Colin, Y. Bénureau, I. Staropoli, Y. Wang, N. Gonzalez, J. Alcami, O. Hartley, A. Brelot, F. Arenzana-Seisdedos, B. Lagane, HIV-1 exploits CCR5 conformational heterogeneity to escape inhibition by chemokines, (2013), Proc. Natl. Acad. Sci. USA, 110: 9475-80.

Scientific or technical background required for work program

A background in real-time microscopy and in the molecular pharmacology of G protein-coupled receptors would be advantageous. The candidate should be able to interact with members of an interdisciplinary partnership and possess excellent interpersonal and scientific communication skills.

Title of the work program 14

Structural and functional study of interactions between myosins and Usher proteins involved in hearing and vision

Description of the work program

Usher syndrome is a genetic disorder characterized by impaired hearing and vision. Mutations in genes encoding the Usher
proteins cause the syndrome and affect structures involved in vision and hearing: photoreceptors and stereocilia, respectively.
Actin-filament stereocilia are grouped into ciliary tufts that detect the sound wave. The proteins involved in Usher syndrome are located in these structures and form complexes. Several cytoplasmic Usher proteins have PDZ domains and PBMs (PDZ binding motifs) such as harmonin and whirlin. The network of interactions between Usher proteins is very complex and forms the Usher interactome. The central nucleus of this interactome consists mainly of the PDZ domains of harmonin and whirlin as well as the protein associated with SANS microtubules. Associated proteins, such as myosin XVa or PDZD7, also interact with Usher proteins.
Among these proteins, I am particularly interested in myosins VIIa and XVa, molecular motors that bind to actin, and their
interactions with whirlin and PDZD7. The expression in bacteria and the purification of different constructions of these proteins are already in place. We have started to characterize the interactions between these proteins by thermophoresis (MST). In addition to MST affinity measurements, structural studies will be carried out on the complexes to define the mode of interaction between the partners. Depending on the complexes, NMR, X-ray crystallography, SAXS (small angle X-ray scattering) and / or electron microscopy are considered.

Objectives:
The student will be involved in biochemical, biophysical and structural studies of these constructions.

Methodologies:
The student will mainly use biochemical and biophysical approaches applied to proteins:
- Cloning
- Expression and purification of recombinant proteins expressed in E. coli
- Biophysical experiments
- Structural biology

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

PDZ Sample Quality Assessment by Biochemical and Biophysical Characterizations.
Caillet-Saguy C, Brûlé S, Wolff N, Raynal B. Methods Mol Biol. 2021;2256:89-124. doi: 10.1007/978-1-0716-1166-1_6. PMID:
34014518

Host PDZ-containing proteins targeted by SARS-CoV-2.
Caillet-Saguy C, Durbesson F, Rezelj VV, Gogl G, Tran QD, Twizere JC, Vignuzzi M, Vincentelli R, Wolff N. FEBS J. 2021 Apr 17.
doi: 10.1111/febs.15881. Online ahead of print. PMID: 33864728

Molecular basis of the interaction of the human tyrosine phosphatase PTPN3 with the hepatitis B virus core protein.
Genera M, Quioc-Salomon B, Nourisson A, Colcombet-Cazenave B, Haouz A, Mechaly A, Matondo M, Duchateau M, König A, Windisch MP, Neuveut C, Wolff N, Caillet-Saguy C. Sci Rep. 2021 Jan 13;11(1):944. doi: 10.1038/s41598-020-79580-9. PMID: 33441627 Free PMC article.

Deciphering the Unexpected Binding Capacity of the Third PDZ Domain of Whirlin to Various Cochlear Hair Cell Partners.
Zhu Y, Delhommel F, Cordier F, Lüchow S, Mechaly A, Colcombet-Cazenave B, Girault V, Pepermans E, Bahloul A, Gautier C, Brûlé S, Raynal B, Hoos S, Haouz A, Caillet-Saguy C, Ivarsson Y, Wolff N. J Mol Biol. 2020 Nov 6;432(22):5920-5937. doi:
10.1016/j.jmb.2020.09.012. Epub 2020 Sep 22. PMID: 32971111

Structural and functional characterization of the PDZ domain of the human phosphatase PTPN3 and its interaction with the human papillomavirus E6 oncoprotein.
Genera M, Samson D, Raynal B, Haouz A, Baron B, Simenel C, Guerois R, Wolff N, Caillet-Saguy C. Sci Rep. 2019 May
15;9(1):7438. doi: 10.1038/s41598-019-43932-x. PMID: 31092861 Free PMC article.

Scientific or technical background required for work program

Expected profile of the candidate:

- Good knowledge of protein biochemistry

- Interest in biochemistry, structural biology and biophysics

Title of the work program 15

New fast powerful sample preparation development on biological samples for electron microscopy

Description of the work program

The Ultrastructural BioImaging Core Facility (UBI) analyses different biological samples on collaborations with research units of the Institut Pasteur and external users. The transmission electron microscopy allows the observation of intracellular ultrastructures after a long and complex embedding process. The core facility acquired a new sample preparation system. This system based on microwaves, reduces drastically the time of preparation and increases the preservation of biological samples. The aim of this project will be to implement this new methodology for transmission electron microscopy (TEM). As a core facility we are working with the whole Institut Pasteur campus the choice of biological samples will be discussed with the student depending of his biological interest. The work is part of a multi-disciplinary project involving partners with expertise in electron microscopy and in host-pathogen interactions.

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Selected publications or patents of the Research Group offering the work program

- Fixation and embedding. Oliver C, Jamur MC. Methods Mol Biol. 2010;588:353-62. doi: 10.1007/978-1-59745-324-0_37. PMID: 20012848
- Rapid microwave fixation of cell monolayers preserves microtubule-associated cell structures. Reipert S, Kotisch H, Wysoudil B, Wiche G. J Histochem Cytochem. 2008 Jul;56(7):697-709. doi: 10.1369/jhc.7A7370.2008. Epub 2008 Apr 14. PMID: 18413652
- Modern approaches for ultrastructural analysis of the zebrafish embryo Nicole L Schieber [1], Susan J Nixon, Richard I Webb, Viola M J Oorschot, Robert G Parton PMID: **20869533** DOI: 10.1016/S0091-679X(10)96018-4

Scientific or technical background required for work program

Scientific background: Good knowledge in cell biology
Technical background: notion on microscopy would be desirable

Title of the work program 16

Optimization of an assay for fast triage of severe / mild Dengue or Covid-19 infected patients

Description of the work program

Early detection of disease is important for improved medical care and treatment. A prognostic biomarker capable of predicting disease severity in Dengue and SARS-CoV-2 infections could help clinicians distinguish between low-risk patients from high-risk patients in need of intensive medical care. Using an in-house assay, we aim to optimize assay conditions to validate the use of a fluorescent enzymatic marker as a potential biomarker for predicting disease severity in Dengue and COVID-19 infections. Optimization involved analyzing for optimum buffer concentration, serum dilution, fluorescent marker concentration, sample storage, measurement of protein concentration & purity.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. Bonam, S. R., Kaveri, S. V., Sakuntabhai, A., Gilardin, L. & Bayry, J. Adjunct Immunotherapies for the Management of Severely Ill COVID-19 Patients. *Cell Rep. Med.* **1**, 100016 (2020).
2. Matangkasombut, P. *et al.* Dengue viremia kinetics in asymptomatic and symptomatic infection. *Int. J. Infect. Dis.* **101**, 90–97 (2020).
3. Poonpanichakul, T. *et al.* Innate Lymphoid Cells Activation and Transcriptomic Changes in Response to Human Dengue Infection. *Front. Immunol.* **12**, 599805 (2021).
4. Torres, J. P. *et al.* Multisystem inflammatory syndrome in children (MIS-C): Report of the clinical and epidemiological characteristics of cases in Santiago de Chile during the SARS-CoV-2 pandemic. *Int. J. Infect. Dis. IJID Off. Publ. Int. Soc. Infect. Dis.* **100**, 75–81 (2020).

Scientific or technical background required for work program

We are looking for students who are motivated and curious. Previous knowledge of bench work would be a plus but is not mandatory.

Title of the work program 17

Structural characterization of herpesvirus glycoproteins bound to antibodies by cryo-EM

Description of the work program

We are interested in understanding how neutralizing antibodies against herpesviruses block viral entry and infection of cells. For this we plan to use a panel of monoclonal antibodies that bind to the glycoprotein B (gB) of herpes simplex virus 1 (HSV-1). The gB is the protein that mediates fusion of the viral and cellular membranes, allowing virus to release the nucleocapsid containing the genetic material into cytosol. It is also a principal viral antigen. By knowing where on gB the antibodies bind, we can gain insight into the neutralization mechanism i.e. do the antibodies block binding of gB to a receptor, to another viral protein, or do they prevent the conformational change that is required for gB to mediate membrane merger.

We have access to a state-of-the-art microscopic facility at the IP that houses 2 Glacios and 1 Titan Krios microscope, allowing us to collect cryo-EM data and perform single particle analyses for structure determination. The advantage of gB bound to antibodies is that the complex is large (>450 kDa) and therefore tractable by cryo-EM. The recombinant proteins needed for the studies, gB and the antibodies, express at levels sufficient for the structural studies. The candidate will learn how to express the recombinant proteins in insect and mammalian cells, purify them (affinity, size exclusion chromatography) and will gain experience in preparation of EM grids and cryo-EM data collection.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1: Light TP, Brun D, Guardado-Calvo P, Pederzoli R, Haouz A, Neipel F, Rey FA, Hristova K, **Backovic M**. *Human herpesvirus 8 molecular mimicry of ephrin ligands facilitates cell entry and triggers EphA2 signaling*. **PLoS Biol.** 2021 Sep 9;19(9):e3001392. doi: 10.1371/journal.pbio.3001392. PMID: 34499637; PMCID: PMC8454987.

2: Vallbracht M, Lötzsch H, Klupp BG, Fuchs W, Vollmer B, Grünewald K, **Backovic M**, Rey FA, Mettenleiter TC. In Vitro Viral Evolution Identifies a Critical Residue in the Alphaherpesvirus Fusion Glycoprotein B Ectodomain That Controls gH/gL-Independent Entry. **mBio.** 2021 May 4;12(3):e00557-21. doi: 10.1128/mBio.00557-21. PMID: 33947756; PMCID: PMC8262866.

3: Vollmer B, Pražák V, Vasishtan D, Jefferys EE, Hernandez-Duran A, Vallbracht M, Klupp BG, Mettenleiter TC, **Backovic M**, Rey FA, Topf M, Grünewald K. *The prefusion structure of herpes simplex virus glycoprotein B*. **Sci Adv.** 2020 Sep 25;6(39):eabc1726. doi: 10.1126/sciadv.abc1726. PMID: 32978151; PMCID: PMC7518877.

4: Vallbracht M, **Backovic M**, Klupp BG, Rey FA, Mettenleiter TC. Common characteristics and unique features: *A comparison of the fusion machinery of the alphaherpesviruses Pseudorabies virus and*

Herpes simplex virus. **Adv Virus Res**. 2019;104:225-281. doi: 10.1016/bs.aivir.2019.05.007. Epub 2019 Jul 3. PMID: 31439150.

5: Vallbracht M, Brun D, Tassinari M, Vaney MC, Pehau-Arnaudet G, Guardado-Calvo P, Haouz A, Klupp BG, Mettenleiter TC, Rey FA, **Backovic M**. *Structure-Function Dissection of Pseudorabies Virus Glycoprotein B Fusion Loops*. **J Virol**. 2017 Dec 14;92(1):e01203-17. doi: 10.1128/JVI.01203-17. PMID: 29046441; PMCID: PMC5730762.

Scientific or technical background required for work program

The candidate must have a background in biochemistry and/or protein chemistry. Some knowledge / coursework on protein structure is required, as well as a keen interest to learn. Virology knowledge is not essential. The candidate will be asked to do some reading prior to joining the project, so that we can get going quickly.

Title of the work program 18

Unravelling the tubulin code

Description of the work program

Microtubules are essential components of the cell cytoskeleton composed of alpha and beta-tubulin heterodimers playing important roles in many cellular processes. Specialisation of tubulin functions is governed by the so-called “tubulin code”. Notably, glutamylation corresponding to the addition of one or more glutamates forming a side chain is one of the most abundant modifications, especially represented in cilia and flagella. *Trypanosoma brucei* is an ideal model to study the tubulin code since their cytoskeleton relies mostly on microtubules and the tubulin code is less complex, facilitating interpretation. Using combined cellular biology, genes knockout and proteomic analysis, our group has identified 2 enzymes belonging to the TTLL family, strongly involved in distinct glutamylation enzymatic activities and structural as well as functionality issues.

An important step is now to map more precisely the glutamylation defects/or modification and get a quantitative analysis of the various proteoforms located at the C-terminus tail of tubulins in *ttll* mutants and wild type cell. To achieve this goal, the aim of this Erasmus project will be to purify flagellar and cytoskeleton tubulins after depolymerization using NaCl or $CaCl_2$ and affinity purification using TOG column on wild type and *ttll* mutants. After *in vitro* tubulin assembly reconstitution, the global profile of tubulin glutamylation and polyglutamylation will be analyzed using LC-MS/MS of intact or fragmented tubulins to provide a detailed and quantitative analysis of these PTM as well as to document for compensation mechanisms provided by the other TTLLs. Following assembly reconstitution, wild type and glutamylation deficient TTLL tubulins will be used to investigate the interaction with *in vitro* expressed molecular motor kinesin and how glutamylated variants affect kinesin processivity along the microtubules. The work is part of a multi-disciplinary project involving partners with expertise in mass-spectrometry (Mass Spectrometry for Biology Unit, Institut Pasteur) and tubulin post-translational modifications (Regulation of Microtubule Dynamics and Function Unit, Institut Curie, Orsay).

At the very proximal region of flagella, the transition zone (TZ) is particularly important because it function as a sieve separating the flagellum from the cell body and is essential to control transport proteins (IFT) injection in the flagellum and its construction. Many proteins are specifically located in this TZ, and mutations in the corresponding genes are responsible for multiple ciliopathies. An apparent decrease in the glutamylation pattern suggest some diversity in this region. To address this issue, a second part of the project will be to enrich TZ microtubules from this region using an established procedure combining tagging of some transition zone proteins, flagellar sonication and affinity purification. Glutamylation profile of these TZ microtubules will be analyzed using mass spectrometry.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. BERTIAUX, E., MALLET, A., ROTUREAU, B and BASTIN, P. (2020). Intraflagellar transport during assembly of flagella of different length in *Trypanosoma brucei* isolated from tsetse flies. **J Cell Sci.** 2020 Sep 23;133(18):jcs248989. doi: 10.1242/jcs.248989.
2. HUET, D., BLISNICK, T., PERROT, S., and BASTIN, P. (2019). IFT25 is required for the construction of the trypanosome flagellum. **J Cell Sci** *132*. doi: 10.1242/jcs.228296
3. BONNEFOY, S.*, Watson, C.M.*, Kernohan, K.D., LEMOS, M., HUTCHINSON, S., Poulter, J.A., Crinnion, L.A., Berry, I., Simmonds, J., Vasudevan, P., O'Callaghan, C., Hirst, R.A., Rutman, A., Huang, L., Hartley, T., Grynspan, D., Moya, E., Li, C., Carr, I.M., Bonthron, D.T., Leroux, M., Care4Rare Canada, C., Boycott, K.M., BASTIN, P.*, and Sheridan, E.G.* (2018). Biallelic Mutations in LRRC56, Encoding a Protein Associated with Intraflagellar Transport, Cause Mucociliary Clearance and Laterality Defects. **Am J Hum Genet** *103*, 727-739.
4. BERTIAUX, E.*, MORGA, B.*, BLISNICK, T., ROTUREAU, B., and BASTIN, P. (2018). A Grow-and-Lock Model for the Control of Flagellum Length in Trypanosomes. **Curr Biol** *28*, 3802-3814 e3803.
5. BERTIAUX, E., MALLET, A., FORT, C., BLISNICK, T., BONNEFOY, S., JUNG, J., LEMOS, M., Marco, S., Vaughan, S., Trepout, S., Tinevez, J.Y., and BASTIN, P. (2018). Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum. **J Cell Biol** *217*, 4284-4297.

   *Highlighted in F1000. Top 5% score of most visible articles on Altmetrics. Comment in: Avasthi, P. **J. Cell Biol** 217:4055-4056.*
6. FORT, C., BONNEFOY, S., Kohl, L., and BASTIN, P. (2016). Intraflagellar transport is required for the maintenance of the trypanosome flagellum composition but not its length. **J Cell Sci** *129*, 3026-3041.
7. SUBOTA, I., JULKOWSKA, D., VINCENSINI, L., REEG, N., BUISSON, J., BLISNICK, T., HUET, D., PERROT, S., SANTI-ROCCA, J., Duchateau, M., Hourdel, V., Rousselle, J.C., Cayet, N., Namane, A., Chamot-Rooke, J., and BASTIN, P. (2014). Proteomic analysis of intact flagella of procyclic Trypanosoma brucei cells identifies novel flagellar proteins with unique sub-localization and dynamics. **Molecular & Cellular Proteomics :** *13*, 1769-1786.
8. ROTUREAU, B., OOI, C.P., HUET, D., PERROT, S., and BASTIN, P. (2014). Forward motility is essential for trypanosome infection in the tsetse fly. **Cell Microbiol** *16*, 425-433.
9. HUET, D., BLISNICK, T., PERROT, S., and BASTIN, P. (2014). The GTPase IFT27 is involved in both anterograde and retrograde intraflagellar transport. **eLife** *3*, e02419.

   Highlighted in F1000.
10. BLISNICK, T., BUISSON, J., ABSALON, S., MARIE, A., Cayet, N., and BASTIN, P. (2014). The intraflagellar transport dynein complex of trypanosomes is made of a heterodimer of dynein heavy chains and of light and intermediate chains of distinct functions. **Mol Biol Cell** *25*, 2620-2633.

Scientific or technical background required for work program

Scientific background: Good knowledge in cell and molecular biology
Technical background: Some expertise in cell culture and/or light microscopy would be desirable.

Title of the work program 19

Temporal dynamics of patterning in the eye of Drosophila

Description of the work program

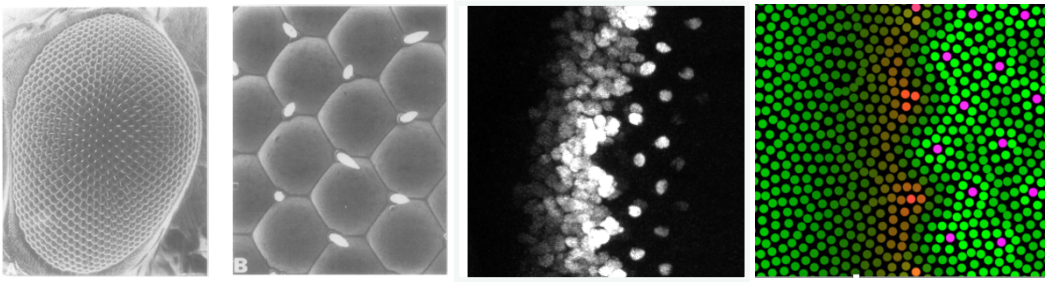
1. General Background

Development proceeds at variable speed in related species. This is exemplified by the biological clock that times the sequential formation of vertebrae which ticks twice faster in the mouse than in humans. The basis underlying such differences in developmental tempo is largely unknown (1). Differences in biochemical reaction speed, notably the rates of protein synthesis and degradation, have been proposed to be responsible for the mouse *vs* man difference in developmental rate (2). More recently, an elegant study proposes that differences in metabolism, specifically the activity of the electron transport chain in mitochondria regulating the NAD+/NADH redox balance, is responsible for the specieis-specific developmental rates in mammals (3). In insects, that do no control their body temperature, the duration of the developmental period also varies with the external temperature. For instance, fruit flies develop twice slower at 18°C than at 25°C. Furthermore, the tempo of insect development also depends on metabolism: ablation of the Insulin Producing Cells in flies reduces energy metabolism and slows down larval development by two-fold (4). The basis underlying these changes in developmental tempo is completely unknown.

2. The flye eye as a model to study the tempo of animal development

The compound eye of insects comprises approx 750 individual light-sensing units, or ommatidia, that are arranged in a crystal-like array (5) (*see figure below*). For each of these units, one photoreceptor cell, R8, acts as a founder cell that sequentially recruits neighboring undifferentiated cells to form the ommatidium. R8 cells emerge along the posterior edge of a differentiation front that sweeps the neuro-epithelium from posterior to anterior during the third instar. Approximately 30 ommatidial rows are produced within 2.5 days, with one new row of regularly spaced R8 cells emerging every 2 hours. The position of the regularly-spaced R8 cells at row n is determined by the R8 cells at row n-1 via a template-based mechanism involving Notch signaling. We recently performed live imaging of this patterning process and discovered that selection of the R8 cells involves a series of gene expression pulses with a 2hr periodicity (our unpublished results). These pulses appear to be intimately linked with the production of R8 cells since each pulse is associated with the emergence of a new row of R8 cells. Therefore, the period of the pulses somehow correlates with the speed of the differentiation front. We are currently

combining experiments with modeling to understand how these pulses of gene expression are generated (*see figure below*).



We propose here to use the developing fly eye as a model system to study the tempo of animal development by studying the period of these pulses and the speed of the differentiation front.

3 Aim and preliminary results

The general aim of this project is to identify the key limiting factors/genes regulating the speed of the differentiation front and/or the period of these pulses in the developing fly eye. To do so, we will screen for candidate genes which are required within the eye epithelium to regulate the speed of the differentiation front. We recently developed a UAS/GAL4 based-assay that will allow us to perform a small-scale RNAi screen (while excluding factors producing systemic perturbations in the developmental timing of the larvae). In parallel, we designed a novel recombination-based tool to measure in vivo the speed of the differentiation front. This tool will be used to characterize the function of the genes identified in our screen. The genes of interest will serve as a starting point to dissect how developmental tempo is regulated in a temperature-dependent manner in the fly eye

References

1. Esibuya and Briscoe 2017 Development PMID:29945985
2. Rayon et al. 2020 Science PMID: 32943498
3. Diaz-Cuadros et al. 2021 bioRxiv https://doi.org/10.1101/2021.08.27.457974
4. Cassidy et al. 2019 Cell 178:980-92
5. Roignant and Treisman 2009 Int J Dev Biol 53:595-604

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

• C. Shard, J. Luna-Escalante and **F. Schweisguth** (2020) Neuralized regulates a traveling wave of Epithelium-to neural stem cell morphogenesis in *Drosophila*. **The Journal of Cell Biology**, 219, e202005035
• L. Couturier, K. Mazouni, F. Corson, **F. Schweisguth** (2019) Regulation of specific *E(spl)-HLH* genes by proneural factors shape output dynamics during bristle patterning in *Drosophila*. **Nature Communications**, 10: 3486
• **F. Schweisguth** and F. Corson (2019) Self-organization in pattern formation. **Developmental Cell,** 49, 659-677 doi: 10.1016/j.devcel.2019.05.019
• D. Henrique and **F. Schweisguth** (2019) Mechanisms of Notch signaling: a simple logic deployed in time and space. **Development**, Feb (46) doi: 10.1242/dev.172148
• M. Trylinski, **F. Schweisguth** (2019) Activation of Arp2/3 by WASp is essential for the endocytosis of Delta only during cytokinesis in *Drosophila*. **Cell Reports**, 28: 1-10
• M. Trylinski, K. Mazouni and **F. Schweisguth** (2017). Intra-lineage fate decisions involve activation of Notch receptors basal to the midbody in *Drosophila* sensory organ precursor cells. **Current Biology**, 27, 2239-47
• F. Corson, L. Couturier, H. Rouault, K. Mazouni and **F. Schweisguth** (2017) Self-organized Notch dynamics generate stereotyped sensory organ patterns in *Drosophila*. **Science** 356, 501, eaai7407

Scientific or technical background required for work program

Background in Genetics and/or Developmental Biology

Title of the work program 20

Structural studies of a bacterial killing secretion machinery by cryo-electron microscopy.

Description of the work program

The student, under the supervision of Dr. F. Gubellini, will work in the Structural Microbiology Unit (Dir. Prof. P. Alzari) on a project coupling cystallography and cryo-electron microscopy to investigate the Type 7 Secretion Systems (T7SSs). These machines, inserted in the bacterial membranes, were discovered initially in *Mycobacterium tuberculosis*, and are present in the majority of Gram-positive bacteria. They secrete different effectors, such as toxins killing other bacteria and proteins modulating the human immune system. We use non-pathogenic model systems (*Bacillus subtilis* and *Corynebacterium glutamicum*) to visualize these complexes, in order to understand their architecture and mechanism. This will allow, in the long term, to design drugs modulating their activity.
The student will perform proteins mutagenesis and purification. She/he will characterize the successful targets with the support of the Biophysics facility at the Institut Pasteur and perform crystallization screening at the Crystallography facility.
During this internship the student will also participate in the cryo-electron microscopy part of the project, including samples preparation, screening, data collect and analysis. The Institut Pasteur has state-of-the-art facilities including different electron microscopes able.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

Tassinari, Doan, Bellinzoni, Chabalier, Ben-Assaya, Martinez, Gaday, Alzari, Cascales, Fronzes, **Gubellini F.** *Central role and structure of the membrane pseudokinase YukC in the antibacterial Bacillus subtilis Type VIIb Secretion System***.** bioRxiv 2020.05.09.085852; doi: https://doi.org/10.1101/2020.05.09.085852

Perry TN, Souabni H, Rapisarda C, Fronzes R, Giusti F, Popot JL, Zoonens M, **Gubellini F.** *BAmSA: Visualising transmembrane regions in protein complexes using biotinylated amphipols and electron microscopy*. Biochim Biophys Acta Biomembr. 2019 Feb 1;1861(2):466-477. doi: 10.1016/j.bbamem.2018.11.004. Epub 2018 Nov 13. PMID: 30444973.

Rapisarda C, Tassinari M, **Gubellini F***, Fronzes R*. *Using Cryo-EM to Investigate Bacterial Secretion Systems*. Annu Rev Microbiol. 2018 Sep 8;72:231-254. doi: 10.1146/annurev-micro-090817-062702. Epub 2018 Jul 13. PMID: 30004822.

Gubellini F, Fronzes R. *Labeling of Membrane Complexes for Electron Microscopy*. Methods Mol Biol. 2017;1635:125-138. doi: 10.1007/978-1-4939-7151-0_7. PMID: 28755367.

Low HH*, **Gubellini F*,** Rivera-Calzada A, Braun N, Connery S, Dujeancourt A, Lu F, Redzej A, Fronzes R, Orlova EV, Waksman G. *Structure of a type IV secretion system.* Nature. 2014 Apr 24;508(7497):550-553. doi: 10.1038/nature13081. Epub 2014 Mar 9. PMID: 24670658.

Scientific or technical background required for work program

The candidate must possess knowledge of classic molecular biology tools (PCR, electrophoresis, DNA purification) and biochemistry techniques (chromatography, SDS-PAGE, Western blotting). Previous experience working with bacteria, membrane proteins and/or electron microscopy will be a plus, but it is not mandatory.

Title of the work program 21

Deciphering HIV-membraneless organelles

Description of the work program

HIV was discovered in 1983 and the reverse transcription (RT) process that characterizes all retroviruses in 1970. However, new notions on the RT were deciphered only recently. Of note, recent studies from our group reveal new spatiotemporal aspects of RT, uncoating (loss of the viral capsid shell) and the topology of HIV pre-integration, maturation and integration. Reverse transcriptase was at the basis of HIV discovery and has been the most exploited antiviral drug target, but inefficient to cure patients. Our recent studies directly highlight the presence of a nuclear RT activity in the nucleus of infected macrophages, revisiting the HIV RT dogma. Of note, inside the host nucleus, newly synthesized viral DNA (vDNA) was found in HIV-1-induced cleavage and polyadenylation specific factor 6 (CPSF6) / serine/arginine-rich splicing factor (SC35) membraneless organelles. These results point to the existence of nuclear RT, contrary to current believes. Other recent studies supported similar conclusions on the spatiotemporal action of the RT. Several speculations can be proposed to explain the presence of these viral/host nuclear structures: (i) to serve as a microenvironment that includes viral and host factors required for the generation of new viral progeny and/or to hide the virus from cellular defense mechanisms; (ii) to serve as a storage of viral genomes.

The aim of our research is untangling the mechanism underlying HIV-1 membraneless organelles biogenesis and their role in viral reverse transcription and replication.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1. pasteur-02548457v1 Blanco-Rodriguez G., Gazi A., B. Monel, Frabetti S., Scoca V., Mueller F., Schwartz O., Krijnse-Locker J., Charneau P., Di Nunzio F Remodeling of the core leads HIV-1 pre-integration complex in the nucleus of human lymphocytes. **J Virol.** 2020 **(Cover JVI 2020)**. doi: 10.1128/JVI.00135-20. Open Access
2. pasteur-03088393v1 Rensen E., Mueller F., Scoca V., Parmar J., Souque P., Zimmer C, Di Nunzio F.Clustering and reverse transcription of HIV-1 genomes in nuclear niches of macrophages, **EMBO J** doi.org/10.1101/2020.04.12.038067 Open Access 2021.
3. pasteur-03214327v1 Scoca V. & Di Nunzio F The HIV-1 capsid: from structural component to key factor for host nuclear invasion. Review V**iruses**, MDPI, 2021, 13 (2), pp.273. (10.3390/v13020273).

4. pasteur-03214326v1 Scoca V. & Di Nunzio F Membraneless organelles restructured and built by pandemic viruses: HIV-1 and SARS-CoV-2. Review **JMCB**, Oxford UP, 2021, ⟨10.1093/jmcb/mjab020⟩
5. pasteur-03214329v1 Scoca V., Louveaux M., Morin R., Ershov D., Tinevez J., Di Nunzio F HIV-induced membraneless organelles orchestrate post-nuclear entry steps (under review) doi.org/10.1101/2020.11.17.385567. **BioRxiv** 2021

6. pasteur-03344209, v1 Guillermo Blanco-Rodriguez, Francesca Di Nunzio. The Viral Capsid: A Master Key to Access the Host Nucleus. **Viruses**, MDPI, 2021, 13 (6), pp.1178. ⟨10.3390/v13061178⟩. ⟨pasteur-03344209⟩ 2021-09-14

Patent Pasteur & NeoVirTech: HIV-ANCHOR

Scientific or technical background required for work program

We are looking for highly motivated and team player individuals with a strong motivation in the following fields:

- virology
- biochemistry
- cell biology
- molecular biology
- image and signal processing

Title of the work program 22

From the embryonic heart loop to structural heart defects in the heterotaxy syndrome

Description of the work program

The shape of an organ is closely related to its function. An important step in cardiac morphogenesis is the looping of the embryonic heart tube to position cardiac chambers relative to each other and establish the double blood flow. In humans, disturbances in this process, which takes place during the third week of gestation, have been associated with severe heart defects in the heterotaxy syndrome.

We have previously demonstrated that the left signal Nodal is required in cardiac precursors for orienting and shaping the embryonic heart loop (Desgrange et al., 2020). *Nodal* mutants develop an heterotaxy syndrome with a spectrum of heart defects. However, the heterogeneity of the heterotaxy phenotype has remained poorly understood. Since *Nodal* mutants at E9.5 display four categories of abnormal heart loops, we reasoned that heterotaxy may be stratified. For example, in a clinical perspective, the final position of the ventricles is considered to reflect the direction of the embryonic heart loop. However, this correlation has never been tested experimentally.

To tackle the challenge of monitoring a specific phenotype at two stages of development in a single individual, we developed a multimodality imaging pipeline, combined with 3D image analysis by virtual reality. The student will contribute to analyse a cohort of mice with heterotaxy, using 3D imaging, quantitative 3D analyses and advanced statistics. Overall, this study, in collaboration with clinicians and computational scientists, is expected to provide novel insights into the emergence of complex congenital defects in the heterotaxy syndrome.

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Laboratory	*Imagine*-Institut Pasteur Unit of Heart Morphogenesis https://research.pasteur.fr/en/team/heart-morphogenesis/

Selected publications or patents of the Research Group offering the work program

Houyel L., Meilhac S.M. *Heart development and congenital structural heart defects.* ***Annu Rev Genomics Hum Genet.*** *202. doi: 10.1146/annurev-genom-083118-015012.*

Desgrange A., Le Garrec J.F., Bernheim S., Holm Bønnelykke T., Meilhac S.M. *Transient Nodal signalling in left precursors coordinates opposed asymmetries shaping the heart loop.* ***Dev Cell.*** *2020. doi: 10.1016/j.devcel.2020.10.008.*

Bernheim S., Meilhac S.M. *Mesoderm patterning by a dynamic gradient of retinoic acid signalling.* ***Philos Trans R Soc Lond B Biol Sci.*** *2020. doi: 10.1098/rstb.2019.0556.*

Desgrange A.*, Lokmer J.*, Houyel L., Meilhac S.M. *Standardised imagign pipeline for phenotyping mouse laterality defects and associated heart malformations, at multiple scales and multiple stages*. ***Dis Models & Mech****, 2019. 12. doi:10.1242/dmm.038356.* (*Co-first author).

Desgrange A., Le Garrec J.F., Meilhac S.M. *Left-right asymmetry in heart development and disease : forming the right loop*. ***Development****, 2018. 145(22). doi: 10.1242/dev.162776.* Revue

Le Garrec J.F., Domínguez J.N.*, Desgrange A.*, Ivanovitch K.D., Raphaël E., Bangham J.A., Torres M., Coen E., Mohun T.J., Meilhac S.M. *A predictive model of asymmetric morphogenesis from 3D reconstructions of mouse heart looping dynamics.* ***eLife****, 2017. 6. pii: e28951* (*equal contribution)

Scientific or technical background required for work program

Strong interest in developmental biology. Previous lab experience.

Title of the work program 23

Role of YfkN, a putative trifunctional nucleotide phosphoesterase in *Enterococcus faecalis*

Description of the work program

The discovery of c-di-AMP in *Bacillus subtilis,* acting as a signaling molecule that couples DNA integrity and sporulation, has extended the number of bacterial second messengers [1] [2] [2]. In *S. aureus* elevation of the c-diAMP hydrolase activity results in an increase sensitivity to beta-lactam antibiotics which suggests an important role in cell wall homeostasis [4]. In addition to its metabolic role, c-di-AMP is also secreted and recognized by the innate immune system of the host [5]. These molecules bind to STING and to the mammalian helicase DDX41 which results in the production of type I interferon [6, 7]. In *Streptococcus agalactiae*, also known as Group B Streptococcus (GBS), a leading cause of invasive infections (pneumonia, septicemia, and meningitis) in the neonate, and a serious cause of mortality or morbidity in adults with underlying diseases, c-di-AMP play also an important role. It is essential for osmotic homeostasis and is secreted when the intracellular concentration becomes high. Outside the bacteria, c-di-AMP is hydrolyzed by an ectonucleotidase anchored to the cell wall CdnP into 2 molecules of AMP [10] which are subsequently hydrolyzed into adenosine and phosphate by NudP [11]. This diminish STING activation by degrading c-di-AMP to promote invasion and organ colonization.

Enterococcus faecalis OG1 has emerged as one of the most common cause of nosocomial infectious disease including urinary tract infections, soft tissue infections, bacteremia, meningitis and endocarditis. In this bacterium CdnP and NudP seem to be contained in a single enzyme YfKN. We propose to study the role of this large enzyme by studying its activity both in vitro and in vivo. We will build different YfkN mutants deleted of either or both activities. The impact on the virulence will be studied in animal model, in collaboration.

1. Witte, G., et al., *Structural biochemistry of a bacterial checkpoint protein reveals diadenylate cyclase activity regulated by DNA recombination intermediates.* Molecular Cell, 2008. **30**(2): p. 167-178.
2. Romling, U., *Great Times for Small Molecules: c-di-AMP, a Second Messenger Candidate in Bacteria and Archaea.* Science Signaling, 2008. **1**(33).
3. Oppenheimer-Shaanan, Y., et al., *c-di-AMP reports DNA integrity during sporulation in Bacillus subtilis.* Embo Reports, 2011. **12**(6): p. 594-601.
4. Corrigan, R.M., et al., *c-di-AMP Is a New Second Messenger in Staphylococcus aureus with a Role in Controlling Cell Size and Envelope Stress.* Plos Pathogens, 2011. **7**(9).
5. Woodward, J.J., A.T. Iavarone, and D.A. Portnoy, *c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response.* Science, 2010. **328**(5986): p. 1703-5.
6. Jin, L., et al., *MPYS Is Required for IFN Response Factor 3 Activation and Type I IFN Production in the Response of Cultured Phagocytes to Bacterial Second Messengers Cyclic-di-AMP and Cyclic-di-GMP.* Journal of Immunology, 2011. **187**(5): p. 2595-2601.
7. Parvatiyar, K., et al., *The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response.* Nature Immunology, 2012. **13**(12): p. 1155-+.

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Selected publications or patents of the Research Group offering the work program

Devaux L, Sleiman D, Mazzuoli MV, Gominet M, Lanotte P, Trieu-Cuot P, Kaminski PA, Firon A. Cyclic di-AMP regulation of osmotic homeostasis is essential in Group B Streptococcus. PLoS Genet. 2018 Apr 16;14(4):e1007342. doi: 10.1371/journal.pgen.1007342. PMID: 29659565; PMCID: PMC5919688.

Devaux L, Kaminski PA, Trieu-Cuot P, Firon A. Cyclic di-AMP in host-pathogen interactions. Curr Opin Microbiol. 2018 Feb;41:21-28. doi: 10.1016/j.mib.2017.11.007. Epub 2017 Nov 21. PMID: 29169058.

Andrade WA, Firon A, Schmidt T, Hornung V, Fitzgerald KA, Kurt-Jones EA, Trieu-Cuot P, Golenbock DT, Kaminski PA. Group B Streptococcus Degrades Cyclic-di-AMP to Modulate STING-Dependent Type I Interferon Production. Cell Host Microbe. 2016 Jul 13;20(1):49-59. doi: 10.1016/j.chom.2016.06.003. PMID: 27414497; PMCID: PMC5382021.

Firon A, Dinis M, Raynal B, Poyart C, Trieu-Cuot P, Kaminski PA. Extracellular nucleotide catabolism by the Group B Streptococcus ectonucleotidase NudP increases bacterial survival in blood. *J Biol Chem*. 2014 Feb 28;289(9):5479-89.

Scientific or technical background required for work program

Bacteriology: Routine technics to isolate and cultivate bacterial strains in medium containing or not antibiotics; bacterial transformation or electroporation with plasmid DNA.

Molecular biology: Chromosomal and plasmid DNA preparation; PCR and molecular cloning.

Biochemistry: protein expression and purification by chromatography affinity.

Title of the work program 24

Molecular mechanism of autophagy in neurodegenerative diseases and cancer

Description of the work program

Autophagy is a major recycling pathway that operates in all eukaryotic cells to maintain cellular homeostasis. It degrades damaged or superfluous cytoplasmic compartments by a de novo formed membrane, termed phagophore. Starvation and cytosolic stresses induce non-selective autophagy that recycles bulk cytoplasm instead of defined cargo to free resources. The molecular switch from selective to nonselective autophagy is not well understood but triggering one or the other pathway is of major interest to develop targeted therapies to treat cancer and neurodegenerative diseases. We are reconstituting autophagy in the test tube using purified components and model membranes to investigate molecular functions of key autophagy factors. We combine this with biochemical, cell biological and structural approaches in vivo using cell culture, primary cells and stem cells to reveal insights into the regulation of autophagy in cells.

The major goal of the project is to investigate how specificity in autophagy is controlled and how cells switch from selective to non-selective autophagy. The project involves reconstitutions of a key step in autophagy from purified and fluorescent labeled proteins on supported lipid bilayers and on liposomes of various sizes. Fluorescence microscopy will be used to investigate membrane binding and fluorescent lifetime imaging (FLIM) will be used to investigate protein-protein interactions on membranes. The structure of the protein on membranes and changes in membrane morphology will be investigated by electron microscopy. Observed interactions will be confirmed by co-immunoprecipitation experiments and by FLIM experiments in cells.
We provide a stimulating working atmosphere and a broad training in various in vitro and in vivo techniques.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

1.Wetzel, L. *et al.* TECPR1 promotes aggrephagy by direct recruitment of LC3C autophagosomes to lysosomes. *Nat Commun* **11**, 2993 (2020).

2.Matscheko, N., Mayrhofer, P., Rao, Y., Beier, V. & Wollert, T. Atg11 tethers Atg9 vesicles to initiate selective autophagy. *Plos Biol* **17**, e3000377 (2019).

3.Rao, Y., Perna, M. G., Hofmann, B., Beier, V. & Wollert, T. The Atg1-kinase complex tethers Atg9-vesicles to initiate autophagy. *Nat Commun* **7**, 10338 (2016).

4.Moparthi, S. B. & Wollert, T. Reconstruction of destruction – in vitroreconstitution methods in autophagy research. *J Cell Sci* **132**, jcs223792 (2018).

Scientific or technical background required for work program

We are looking for student who are interested to work at the intersection of cell biology and physical biology with experience in handling humsn cell culture, in microscopy or in recombinant protein expression. We provide a broad training in all these areas either from scatch or to deepen preexisting knowledge and experience.

Title of the work program 25

Role of microRNAs in the type I interferon-dependent modulation of the human T cell response

Description of the work program

We study the immunomodulatory activity of the type I interferon family (IFNα/β) on the human T cell immune response in healthy donors and patients with multiple sclerosis (MS). This chronic autoimmune and inflammatory disease targets the central nervous system, causing axonal demyelination, neurodegeneration and gradual physical disabilities. The most common form of the disease is relapsing-remitting (RRMS), which is commonly treated by IFNβ as a first-line therapy. Our goals are to uncover immune signatures that could help to determine disease or treatment response biomarkers and to identify cellular and molecular mechanisms involved in the immunopathogenesis of the disease.

MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally regulate hundreds of genes and gene networks involved in several vital biological processes, including the immune response. They mainly act by binding to the 3' untranslated region of messenger RNAs, thereby inducing mRNA cleavage and gene silencing or mRNA destabilization and reduced gene expression. Cellular miRNA signatures have been also proposed in health and disease according to their cell type- and context-dependent expression. Based on our small RNA-seq data, we have identified a set of miRNAs that are modulated by IFNα/β in human CD4+ T cells. Some of them are predicted to regulate CD4+ T cell activation and function. We have also identified miRNAs that are involved in the regulation of the IFNα/β signaling pathway. One objective of the project is to validate the IFN-dependent modulation of miRNAs candidates by specific RT-qPCR and to study if this modulation is immune cell type- and/or activation cell state-dependent. A second objective is to study the impact of selected miRNAs in the regulation of the CD4+ T cell response by performing mechanistical assays. Time permitting, these studies may be extended to RRMS patients at different stages of the disease.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program

- Devi-Marulkar P, Moraes-Cabe C, Campagne P, Corre B, Meghraoui-Kheddar A, Bondet V, Llibre A, Duffy D, Maillart E, Papeix C, Pellegrini S, Michel F. Altered Immune Phenotypes and HLA-DQB1 Gene Variation in Multiple Sclerosis Patients Failing Interferon β Treatment. *Front Immunol.* 2021 May 25;12:628375. doi: 10.3389/fimmu.2021.628375. eCollection 2021. PMID: 34113337

- Rubino E, Cruciani M, Tchitchek N, Le Tortorec A, Rolland AD, Veli Ö, Vallet L, Gaggi G, Michel F, Dejucq-Rainsford N, Pellegrini S. Human Ubiquitin-Specific Peptidase 18 Is Regulated by microRNAs via the 3'Untranslated Region, A Sequence Duplicated in Long Intergenic Non-coding RNA Genes Residing in chr22q11.21. *Front Genet.* 2021 Feb 3;11:627007. doi: 10.3389/fgene.2020.627007. eCollection 2020. PMID: 33633774

- Li Z, Rotival M, Patin E, Michel F, Pellegrini S. Two common disease-associated TYK2 variants impact exon splicing and TYK2 dosage. *PLoS One*. 2020 Jan 21;15(1):e0225289. doi: 10.1371/journal.pone.0225289. eCollection 2020. PMID: 31961910

- Azébi S, Batsché E, Michel F, Kornobis E, Muchardt C. Expression of endogenous retroviruses reflects increased usage of atypical enhancers in T cells. *EMBO J.* 38(12):e101107. Epub 2019 May 8. PMID: 31068361 DOI: 10.15252/embj.2018101107

- U. Govender, B. Corre, Y. Bourdache, S. Pellegrini and F. Michel. Type I interferon-enhanced IL-10 expression in human CD4 T cells is regulated by STAT3, STAT2, and BATF transcription factors. *J. Leukoc. Biol*. 101(5):1181-1190. Epub 2017 Feb 27. PMID: 28242623 DOI :10.1189/jlb.2A0416-187RR

- B. Corre, J. Perrier, M. El Khouri, S. Cerboni, S. Pellegrini and F. Michel. Type I interferon potentiates T-cell receptor mediated induction of IL-10-producing $CD4^+$ T cells. *Eur. J. Immunol.,* 43(10):2730-40. Epub 2013 Jul 15. DOI: 10.1002/eji.201242977

Scientific or technical background required for work program

An experience in the regulation of gene and microRNA expression, in T cell adaptive immunity and flow cytometry will be a strong advantage.