Erasmus+

Institut Pasteur

Projects 2019-2020

RESEARCH CENTRE

Legal name: **Institut Pasteur**

Address: **25-28 rue du Dr. Roux, 75724 Cedex 15, PARIS**

Country: **France**

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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 Institut Pasteur, Paris, France have proposed 31 projects for Erasmus internships (see following pages). In addition, two projects have been presented from the International Pasteur Network, and are mainly reserved for PhD level students to be discussed by the candidates with their University).
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) at Institut Pasteur, Paris

- **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
Partially subsidized by the Institute
- **Additional salary**   **X** YES   ☐ NO
  Institut Pasteur Paris offers an additional salary of approximately 550 euros/month, which is paid by the host lab (3.75 euros/hour, 7 hours/working day).

NOTE: internship conditions at the **International Pasteur Network** may vary and have to be discussed directly with the host lab.

Title of the work program 1

NMR structural study of pentameric ligand gated ion channels

Description of the work program

Our major subject concerns pentameric ligand gated ion channels (pLGICs, including nicotinic, GABAA and glycine receptors) that mediate neuronal communication. pLGICs are involved in many diseases, including nicotinic addiction, epilepsy, neurodegenerative and psychiatric diseases such as Alzheimer and Parkinson diseases. They are the target of important classes of therapeutic and addictive drugs including general anesthetics, anxiolytics, anti-vomiting and anti-smoking compounds. Therefore, understanding the molecular mechanisms involved in functioning of these channel-receptors, as well as their regulation/deregulation by therapeutic and addictive drugs is currently a matter of intense research activity by academia and industry. Following the discovery of a bacterial ancestor of pLGICs called GLIC in our lab, we collected a set of structural (using X-ray crystallography and fluorescence) and functional (using electrophysiology and surface plasmon resonance) data allowing us to propose a mechanism of activation for a member of this family of channels.

Our project aims at understanding the fundamental allosteric mechanisms mediating the signal transduction of pLGICs. NMR spectroscopy coupled to specific labelling is well-suited to characterize transiently populated conformations as well as the transitions between different conformations in solution. Using the model system GLIC, we have developed specific labeling and performed NMR experiments, to monitor conformational and dynamics modifications of the GLIC receptor depending on its activation state (as a function of pH or in ligand-bound form). We benefit from a large panel of Cys -mutants of GLIC to choose appropriate positions. Fluorine- and 13C-S-methylthiocysteine (MMTS-13C) labelled GLIC mutants will be expressed in E. coli and purified in order to perform 19F and 13C NMR spectroscopy, respectively. In addition, GLIC mutants will be investigated by Xray crystallography to search for conformations. In parallel, we will also use NMR approaches to screen small compounds targeting the ligand-binding domain of the nicotinic acetylcholine receptors. We aim at discover allosteric modulators specifically targeting α5-containing nAChRs and we have recently develop fragment-screening on soluble AChBP receptor, a good model for the ligand-binding domain of the nicotinic acetylcholine receptors. NMR characterization of complexes formed by AChBP and known modulators will be performed as a first step toward a high-throughput screening of fragment bank.

Tutor/supervisor

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

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.

Structural plasticity of the HHD2 domain of whirlin.
Delhommel F, Cordier F, Saul F, Chataigner L, Haouz A, Wolff N.
FEBS J. 2018 Oct;285(20):3738-3752. doi: 10.1111/febs.14614. Epub 2018 Sep 5.

Structural Characterization of Whirlin Reveals an Unexpected and Dynamic Supramodule Conformation of Its PDZ Tandem.
Delhommel F, Cordier F, Bardiaux B, Bouvier G, Colcombet-Cazenave B, Brier S, Raynal B, Nouaille S, Bahloul A, Chamot-Rooke J, Nilges M, Petit C, Wolff N.
Structure. 2017 Nov 7;25(11):1645-1656.e5. doi: 10.1016/j.str.2017.08.013. Epub 2017 Sep 28.

Regulation of the Human Phosphatase PTPN4 by the inter-domain linker connecting the PDZ and the phosphatase domains.
Caillet-Saguy C, Toto A, Guerois R, Maisonneuve P, di Silvio E, Sawyer K, Gianni S, Wolff N.
Sci Rep. 2017 Aug 11;7(1):7875. doi: 10.1038/s41598-017-08193-6.

Molecular Basis of the Interaction of the Human Protein Tyrosine Phosphatase Non-receptor Type 4 (PTPN4) with the Mitogenactivated Protein Kinase p38γ.
Maisonneuve P, Caillet-Saguy C, Vaney MC, Bibi-Zainab E, Sawyer K, Raynal B, Haouz A, Delepierre M, Lafon M, Cordier F, Wolff N.
J Biol Chem. 2016 Aug 5;291(32):16699-708. doi: 10.1074/jbc.M115.707208. Epub 2016 May 31.

Scientific or technical background required for work program

The student will produce constructions of GLIC and/or nAchBP. He will be involved in biochemical, biophysical and structural studies of these constructions. Constructs will be mainly produced for NMR studies.

Expected profile of the candidate:
- General knowledge of protein biochemistry
- Interest in structural biology and biophysics

Title of the work program 2

Holographic light stimulation of the cochlea to study auditory processing

Description of the work program

Processing of auditory information in the brain is complex because information not only flows from the auditory periphery to the central nervous system but also from the brain to the ear. As a result, efferent neuronal signals can modulate the mechanical properties of the cochlea. Ideally, we would like to know the cochlear output precisely to study its effect on neural representations. However, because cochlear mechanics and neuronal processing are reciprocally coupled through mechanoelectrical feedback, it will require specific tools to uncouple them and to decode the transformation of complex acoustic stimuli by the brain.

The aim of this project is to study how information about sound frequency is propagated from the auditory periphery to the cortex. To understand how sound features are encoded in the brain we need to vary specific parameters of the input and measure how it affects neuronal firing. Recent progress in optogenetics have allowed to activate neuronal circuits precisely. Here we will use these tools to control the cochlear output and realize the first optogenetic activation of cochlear hair cells *in vivo*. Optical methods allow to focalize the beam of a laser onto several cellular targets and rapidly update the temporal pattern of stimulation. The student will design a setup based on holographic light patterning to be able to stimulate simultaneously but independently single hair cells with millisecond precision.

Tutor/supervisor

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

1. Barral J, Wang XJ, and Reyes AD (2019). Propagation of temporal and rate signals in cultured multilayer networks. **Nature Communications** 10(1): 3969
2. Barral J, Jülicher F, and Martin P (2018). Friction from transduction channels' gating affects spontaneous hair-bundle oscillations. **Biophysical Journal** 114(2):425-436
3. Barral J and Reyes AD (2017) Optogenetic stimulation and recording of primary cultured neurons with spatiotemporal control. **Bio-Protocol** 7(12): e2335
4. Barral J and Reyes AD (2016). Synaptic scaling rule preserves excitatory/inhibitory balance and salient neuronal network dynamics. **Nature Neuroscience** 19 :1690-1696
5. Barral J and Martin P (2012). Phantom tones and suppressive masking by active nonlinear oscillation of the hair-cell bundle. **P.N.A.S.** 109 : E1344-51
6. Barral J and Martin P (2011). The physical basis of active mechanosensitivity by the hair-cell bundle. **Cur. Op. in Otolaryngology and Head and Neck Surgery.** 19 : 369-375
7. Barral J, Dierkes K, Lindner B, Jülicher J, and Martin P (2010). Coupling a sensory hair-cell bundle to cyber clones enhances nonlinear amplification. **P.N.A.S.** 107 : 8079-8084

Scientific or technical background required for work program

We want to study how sensory information is encoded in the brain. We are looking for individuals with enthusiasm, curiosity, ambition, and happy to work in a team.

Experience in the following areas: *in vivo* electrophysiology, 2-photon imaging, optogenetics, microscopy, computer science is a plus.

Title of the work program 3

Integrative structural biology studies of DNA replication molecular machines

Description of the work program

All forms of life have evolved large protein complexes, named the replisomes, which carry out DNA replication, starting at the replication origin. The replisome contains several enzymatic activities, such as helicase, primase and DNA polymerase and creates a replication fork to duplicate both the leading and lagging strand. Replicative DNA polymerases (DNAPs) are highly processive and accurate enzymes, responsible for duplicating the genome, and are key actors of the replisome. PolD is an archaeal replicative DNA polymerase (DNAP) made of a proofreading exonuclease subunit (DP1) and a larger polymerase catalytic subunit (DP2). Our group has reported the individual crystal structures of the DP1 and DP2 catalytic cores, thereby revealing that PolD is an atypical DNA polymerase, which has all functional properties of a replicative DNA polymerase but with the catalytic core of a RNA polymerase (RNAP). Recently, we determined the DNA-bound cryo-EM structure of the heterodimeric DP1-DP2 PolD complex from Pyrococcus abyssi, revealing a unique DNA-binding site. PolD has been shown to be essential for cell viability, and is widely distributed among Archaea, being present in all four major superphyla: Euryarchaeota (including the methanogenic human symbionts), the emerging Asgard superphylum, DPANN, and TACK (only absent from Crenarchaeota). PolD has been shown to interact with several replication factors.

Using a multi-disciplinary approach combining electron microscopy, X-ray crystallography, activity assays and various protein-protein interactions measurements, we aim to resolve the molecular mechanisms of DNA replication by PolD in the context of the replisome, on the DNA fork. The Unit for Structural Dynamics of Macromolecules at the Pasteur Institute is a well published and highly dynamic research environment that applies a multidisciplinary research strategy including structural biology, biochemistry, and biophysics to address fundamental questions about the molecular mechanisms of DNA replication.

The laboratory is located at the Pasteur Institute in Paris that has an outstanding range of facilities and instrumentation, including a state-of-the-art Titan Kryos equipped with a K3 camera, and several screening microscopes. Additional resources for specimen preparation and image processing are in place. No prior experience in X-ray crystallography and Cryo-electron microscopy is required, the host lab will provide full training to the successful candidate.

Tutor/supervisor

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

The Extended 'Two-Barrel' Polymerases Superfamily: Structure, Function and Evolution. Sauguet L , J. Mol. Biol. 2019 doi : 10.1016/j.jmb.2019.05.017

Structure of the DP1-DP2 PolD complex and its implications for the evolutionary history of DNA and RNA polymerases. Raia P, Carroni M, Henry E, Pehau-Arnaudet G, Brûlé S, Béguin P, Henneke G, Lindahl E, Delarue M, Sauguet L, PLoS Biology 2019 17(1) :e3000122

An updated structural classification of replicative DNA polymerases. Raia P, Delarue M, Sauguet L, Biochem. Soc. Transactions 2019 47(1)239-249

Shared active site architecture between archaeal DNA polymerase D and multisubunit RNA polymerases revealed by X-ray crystallography. Sauguet L, Raia P, Henneke G, Delarue M Nature communications 2016 (7) 12227

Scientific or technical background required for work program

The successful candidate should have a background in molecular biology and/or biochemistry. No prior experience in X-ray crystallography and Cryo-electron microscopy is required, the host lab will provide full training to the successful candidate, but the candidate should be highly motivated in learning structural biology.

Title of the work program 4

Identification of the substrates of transglutaminases using Click-it chemistry

Description of the work program

Transglutaminases (TGs) are a family of structurally and functionally related proteins that catalyze the Ca^{2+}-dependent posttranslational modification of proteins by introducing covalent bonds between free amine groups (e.g., protein- or peptide-bound lysine) and carboxamide groups of peptide-bound glutamines. The best studied is the ubiquitously expressed TG2, and several dozens of TG2 substrates have been identified. Much less is known about TG1 and TG6 substrates, that show more tissue-specific expression, and are in particular expressed in skin and neurons, respectively. Even for TG2 substrates, the exact glutamine residue(s) to which TG2 attaches an amine group remains to be identified in most cases.

Transamidation by TGs have many physiological consequences, which are likely at least in part mediated by the post-translational modification of their target proteins. For instance TG2 is over expressed in many cancer tissues, and its activation favors tumor progression. TG1 is highly upregulated in ischemic brain and genetic mutations in TG6 lead to a rare form of ataxia called spinocerebellar ataxia (SCA) 35. However, the molecular details between the transamidation activity and the physiological outcomes are largely unknown. The purpose of this project is to use a proteomic approach coupled to Click-it chemistry to identify glutamine acceptors of transamidation reactions catalyzed by TG1, TG2 and TG6. This will be a first step towards a better understanding of effect of the activation of these TGs on cell physiology. If time permits, the student will also initiate the investigation of the functional consequences of transamidation of one of the most prominent TG2 target.

During this internship the student will be trained in a variety of techniques: tissue culture, western blots, click-it chemistry, immunofluorescence imaging, cloning, protein production and purification. He/she will also work closely with the mass-spectrometry facility for the analysis of the data

Tutor/supervisor

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

Maffei, B., M. Laverriere, Y. Wu, S. Triboulet, S. Perrinet, M. Duchateau, M. Matondo, R.L. Hollis, C. Gourley, J. Rupp, J.W. Keillor, and A. Subtil. 2019. Infection-driven activation of transglutaminase 2 boosts glucose uptake and hexosamine biosynthesis. *bioRxiv*:634501.

Scientific or technical background required for work program

None, although past experience at working at the bench will be welcome.

Title of the work program 5

Post-transcriptional regulation of interleukin 10 expression by microRNAs in human CD4+ T cells

Description of the work program

We study the immunomodulatory activity of type I interferon family (IFNα/β) on the T cell adaptive immune response in healthy donors and patients with multiple sclerosis (MS). This chronic immune-mediated disease targets the central nervous system and leads to progressive neuroaxonal demyelination, neurodegeneration and gradual physical disabilities. Type I interferon, IFNβ, is commonly prescribed as a first-line treatment of the relapsing-remitting form of MS. However around 30% of patients do not respond to the therapy. Our major goal is to uncover cellular and molecular signatures that could help as prognostic biomarkers of IFN responsiveness in MS patients. In this regard, a favorable outcome of the disease has been associated with increased level of the potent anti-inflammatory cytokine, IL-10, and a decrease in pro-inflammatory cytokines. We have shown that IFNα/β potentiates IL-10 expression in human CD4+ T cells stimulated by the T cell receptor (TCR) and promotes development of Foxp3$^-$ type 1 regulatory-like cells (Tr1-like cells). Tr1 cells exert a critical function by contributing to the maintenance of immune homeostasis and tolerance to self-antigens. By dissecting the contributions of TCR and IFN pathways in the potentiation of IL10 expression, we have recently uncovered regulatory transcription factors. We plan to further study IL-10 regulation at the post-transcriptional level by non-coding microRNAs in CD4+ T cells. Based on RNA-seq data, we have identified microRNAs candidates that potentially control the expression of IL10 and of regulators of the IFN response. A number of these microRNAs are predicted to be distinctly activated in different immune cell types. Expression and impact of miRNAs candidates will be gained using primary CD4+ T cells from healthy donors and T cell line models. Insights from this study may be translated to multiple sclerosis patients, taking advantage of the project that we are developing.

(https://research.pasteur.fr/en/program_project/milieu-interieur-labex/).

Tutor/supervisor

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

- Azébi S, Batsché E, Michel F, Kornobis E, Muchardt C. 2019. Expression of endogenous retroviruses reflects increased usage of atypical enhancers in T cells. *Embo J.* May 8. pii: e101107. Doi 10.15252/embj.2018101107. PMID: 31068361

- U. Govender, B. Corre, Y. Bourdache, S. Pellegrini and F. Michel. 2017. Type I interferon-enhanced IL-10 expression in human CD4 T cells is regulated by STAT3, STAT2, and BATF transcription factors. *J. Leukoc. Biol.* Doi :10.1189/jlb.2A0416-187RR

- Zhang X., Bogunovic D., Payelle-Brogard B., Francois-Newton V., Speer S, Yuan C, Volpi S, Li Z, Sanal O, Mansouri D, Tezcan I, Rice GI, Chen C, Mansouri N, Mahdaviani S, Itan Y, Boisson B, Okada S, Zeng L, Wang X, Jiang H, Liu W, Han T, Liu D, Ma T, Wang B, Liu M, Liu J, Wang QK, Yalnizoglu D, Radoshevich L, Uzé G, Gros P, Rozenberg F, Zhang S-Y, Jouanguy E, Bustamante J, García-Sastre A, Abel L, Lebon P, Notarangelo L, Boisson-Dupuis S, Crow YJ, Casanova J-L and Pellegrini S. 2015. Human intracellular ISG15 prevents IFN-α/β over-amplification and auto-inflammation. *Nature,* 517: 89-93

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

- Z. Li, M. Gakovic, J. Ragimbeau, M-L Eloranta, L Rönnblom, F Michel and S Pellegrini. 2013. Two rare disease-associated Tyk2 variants are catalytically impaired but signaling competent. *J. Immunol.,* 190(5):2335-44.

- Francois-Newton V., Livingstone M., Payelle-Brogard B., Uzé G., and Pellegrini S. 2012. USP18 establishes the transcriptional and anti-proliferative interferon α/β differential. *Biochem. J.* 446, 509-516.

- Francois-Newton V., de Freitas Almeida G., Payelle-Brogard B., Monneron D., Pichard-Garcia, L. Piehler, J., Pellegrini S., and Uzé G. 2011. USP18-based negative feed-back control is induced by Type I and Type III Interferons and specifically inactivates interferons a response. *PLoS ONE* 6(7):e22200.

Scientific or technical background required for work program

An experience in molecular biology, regulation of gene expression, microRNA, and in cellular immunology, notably in T cell adaptive immunity would be a strong advantage.

Title of the work program 6

Towards the mechanism of recombination-independent DNA homology search and recognition

Description of the work program

We are interested in discovering and characterizing new mechanisms that organize DNA in the nucleus. We are especially interested in understanding genetic phenomena that involve interactions between apparently intact segments of homologous DNA. Such processes are remarkably ubiquitous, yet their molecular nature remains one of the most enigmatic unanswered questions in biology.

Using the premeiotic phenomenon of Repeat Induced Point mutation (RIP) in the fungus Neurospora crassa as a model system, we discovered that DNA homology can be recognized using a fundamentally new approach, by which long segments of double-stranded DNA (dsDNA) are compared to one another as arrays of interspersed base-pair triplets. Importantly, this process does not require the function of RecA proteins. The ability of RIP to detect any two identical DNA segments placed at the arbitrary sites in the genome suggests that RIP involves an exhaustive, "genome-by-genome" homology search. Therefore, this search process must be very efficient. Intriguingly, we have found that RIP mutation can be mediated by an epigenetic pathway that includes the SUV39 methyltransferase DIM-5, which catalyzes trimethylation of histone H3 lysine-9 residues. Thus, we have proposed that SUV39 methyltransferases can be recruited to repetitive DNA ab initio in response to homologous dsDNA-dsDNA interactions. In our ongoing work, in collaboration with the laboratory of Tom Hammond, we have found that the same homology-recognition principles that direct RIP mutation are also applicable to Meiotic Silencing by Unpaired DNA (MSUD). These new results (i) reveal the role of a recombination-independent homology-directed process in guiding the expression of small interfering RNAs, (ii) suggest that homologous chromosomes can be matched during meiosis by a mechanism that operates on intact DNA double helices, and (iii) raise an intriguing possibility that the revealed recombination-independent mechanism may represent a general, perhaps even fundamental, mode of DNA homology search and recognition.

Current & prospective projects in the lab include (i) discovering molecular factors that recognize repetitive DNA in N. crassa, (ii) elucidating the mechanism of recruitment of DIM-5 to repetitive DNA in N. crassa, (iii) further understanding the DNA homology requirements for RIP and MSUD in N. crassa, and (iv) using a range of imaging techniques to characterize the state of chromatin in the premeiotic nuclei of N. crassa.

Tutor/supervisor

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

Rhoades N, Cecere G, Hammond T, Gladyshev E (2019). Recombination-independent recognition of DNA homology for meiotic silencing in Neurospora crassa. bioRxiv, 573907. doi: 10.1101/573907 [PREPRINT]

Mazur AK, Gladyshev E (2018). Partition of Repeat-Induced Point Mutations Reveals Structural Aspects of Homologous DNA-DNA Pairing. Biophysical Journal 115: 1-11. doi:10.1016/j.bpj.2018.06.030.

Gladyshev E (2017). Repeat-Induced Point Mutation and Other Genome Defense Mechanisms in Fungi. Microbiology Spectrum 5(4). doi: 10.1128/microbiolspec.FUNK-0042-2017. [review]

Gladyshev E, Kleckner N (2017). DNA sequence homology induces cytosine-to-thymine mutation by a heterochromatin-related pathway. Nature Genetics 49(6): 887-894. doi: 10.1038/ng.3857.

Gladyshev E, Kleckner N (2017). Recombination-independent recognition of DNA homology for repeat-induced point mutation. Current Genetics 63(3): 389-400. doi: 10.1007/s00294-016-0649-4. [review]

Gladyshev E, Kleckner N (2016). Recombination-independent recognition of DNA homology for Repeat-Induced Point mutation (RIP) is modulated by the underlying nucleotide sequence. PLOS Genetics, 12: e1006015, doi: 10.1371/journal.pgen.1006015.

Gladyshev E, Kleckner N (2014). Direct recognition of homology between double helices of DNA in Neurospora crassa. Nature Communications, 5: 3509, doi: 10.1038/ncomms4509.

Scientific or technical background required for work program

Basic microbiology & molecular cloning techniques

Title of the work program 7

Modeling Alzheimer in a neural circuit of Drosophila larva

Description of the work program

Neurodegenerative disease like Alzheimer, Parkinson, Huntington disease etc., are a major health issue affecting millions of people worldwide (1). Yet the available treatments are limited and address the disease symptoms. This is because the neurobiological bases of the disease are poorly understood, especially at the neural circuit level. The failure in models for neurodegenerative diseases partially stems from the methodological approaches designed to address them. On one hand, the lack of synaptic and single cell resolution in studying the structural and functional changes of neuronal circuits impairs the understanding of the evolution of the disease and the detailed analysis of the effect of the disease on the neural circuit computations. On the other, the limited number of individuals tested, which can be as low as few dozen mice, prevents the proper sampling of phenotypic behavioural diversity in a population. These two effects combined strongly contribute to the high rate of failure in searching for drugs that might have a significant effect on the neurodegenerative diseases such asAlzheimer.

Drosophila as a model system offers many advantages to make rapid progress in elucidating the mechanisms underlying neurodegenerative disease in a cost-effective manor. The Drosophila neurons are similar to human neurons in terms of their shape, synaptic intercommunications and biochemical signatures. In addition, 70% of disease related genes in humans are found in Drosophila, allowing fly human disease models to be generated efficiently. The powerful genetic tools available in Drosophila combined with the rapid reproductive cycle makes it possible to manipulate both genetic and neuronal activity in a cell-type specific manner and quickly detect changes in behaviour. In addition to the numerical simplicity and tractability of its nervous system, the advances in optical neurophysiology allow the analysis of physiological properties of neurons and their functional relationships. In addition, in the larval Drosophila, an electron microscopy (EM) images of the entire nervous system have been generated and circuits can be mapped with synaptic resolution. Finally, various experimental approaches have demonstrated, the possibility of deciphering the properties and function of small neural circuits (2-7), the possibility to quantitatively characterize the larva dynamics by generating robust dictionary of behaviour (8-10) , to build a correlation map between neural activity and behaviour (11) the possibility of learning (12, 13) and the possibility of performing robust operant learning (unpublished results). Hence, the Drosophila larva shows the promise to be a game changer if established as a model for neurodegenerative diseases. The Drosophila larva allows bridging the two main gaps in the study (and search for cure): sampling of hundreds of thousands of individuals in few months (2-3 months) and accessing synaptic resolution of the selected circuits.

The goal of this project is to prove our intuition by showing within the next three years, that the larva can be used as a neurodegenerative disease model and that the entire data processing can be fully automated. We will focus this project on Alzheimer Disease (AD), as the phenotypes in Drosophila AD models have been well-described (14, 15) and preliminary data already hints at detectable patterns in behaviour induced by the disease.

We have established a methodological approach that combines the computational power of an automated machine learning-based behavioural analysis method with the recently emerged model system for circuit studies: the Drosophila larva that allow us to detect even the smallest behavioural phenotypic changes upon disruption of neuronal circuit function (8). We propose to establish a new model system for neurodegenerative and neuropsychiatric disorder that leverage

the advantages of Drosophila larva as a model system for circuit studies (2, 4, 16, 17)) to a probabilistic approach linking behavioural sequence generation to the disease.

1. C. L. Masters et al., Nat Rev Dis Primers. 1, 15056 (2015). 2. T. Jovanic et al., Cell. 167, 858–870.e19 (2016). 3. T. Jovanic et al., Current Biology (2019). 4. M. F. Zwart et al., Neuron. 91, 615–628 (2016). 5. A. Fushiki et al., eLife. 5, e13253 (2016).6. E. S. Heckscher et al., Neuron. 88, 314–329 (2015). 7. T. Ohyama et al., Nature. 520, 633–639 (2015).8. T. Jovanic, J.-B. Masson, J. W. Truman, M. Zlatic, bioRxiv, 1–22 (2017).9. J. T. Vogelstein et al., Science. 344, 386–392 (2014). 10. T. Ohyama et al., PLoS ONE. 8, e71706 (2013).11. R. Gepner, M. Mihovilovic Skanata, N. M. Bernat, M. Kaplow, M. Gershow, eLife. 4 (2015), doi:10.7554/eLife.06229.12. T. Saumweber et al., Nat Commun. 9, 1104 (2018). 13. M. Schleyer, D. Miura, T. Tanimura, B. Gerber, eLife. 4, 6 (2015). 14. S. D. Mhatre eal., Dis Model Mech. 7, 373–385 (2014).
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Selected publications or patents of the Research Group offering the work program

- J.-B. Masson et al, Mapping neurons and brain regions underlying sensorimotor decisions and sequences in Drosophila (under review)

- El Beheiry et al , Virtual Reality: Beyond Visualisation, J. Mol. Biol., vol. 431, issue 4, (2019)

- Jovanic, T et al. Neural Substrates of Drosophila Larval Anemotaxis. *Curr. Biol.*, 29, 4, (2019)
- Floderer* C et al , Single molecule localisation microscopy reveals how HIV-1 Gag proteins sense membrane virus assembly sites in living host CD4 T cells, Sci. Rep.,8, No 16283, (2018)

- Jovanic T et al. Competitive Disinhibition Mediates Behavioral Choice and Sequences in Drosophila. Cell. Oct 20;167(3) (2016)

- Knight S. C. et al , Dynamics CRISPR-CAS9 genome interrogation in living cells., Science vol 350, Issue 6262, p823-826 (2015).

Scientific or technical background required for work program

2 possible profiles (non-mutually exclusive):
- Biology, Neuroscience & Genetics
- Computational biology, statistical physics, applied mathematics

Title of the work program 8

Investigation of actin- and membrane-mediated interactions in the formation of tunneling nanotubes (TNTs)

Description of the work program

The way in which cells interact with each other is a topic that has fascinated biologists for many centuries. Our group is interested in a novel type of cell-to-cell interaction that has recently been characterized as a direct connection between cells. This connection is known as a Tunneling Nanotube, or TNT. TNTs are direct connections between cells found in many cell types and contexts. Unlike other cellular protrusions (e.g., filopodia), TNTs connect the cytoplasm of distant cells. TNTs vary in diameter and can extend up to 100 microns in length. These dynamic structures selectively transfer cellular cargo such as cytoplasmic molecules, plasma membrane components, vesicles, and even large organelles such as mitochondria. In addition, TNTs have been shown to be "hijacked" by various pathogens such as bacteria and viruses in order to transfer between cells. In our group, we have spent the last decade setting up the tools necessary to identify and characterize TNTs in culture and have demonstrated that they play an important role in the intercellular transfer of misfolded and aggregated proteins involved in neurodegenerative diseases. The ability of TNTs to transfer cargo between cells may therefore be relevant to understand essential biological processes such as development, pathological response, cancer, tissue regeneration, and electrical signal transmission. However, the underlying physical and biological mechanisms behind TNT formation are unknown.

In standard cell culture, TNT formation with cell neighbors is random and it is challenging to differentiate TNTs from visually similar filopodia, since no molecular marker yet exists. Utilizing micropatterning techniques to precisely control intercellular distances and cellular densities, we identified physical parameters and distance thresholds over which the formation of functional TNTs is promoted. By directly controlling TNT growth between positioned cells, the involvement of actin, actin-regulating proteins and actin-membrane connectors can now be fully ascertained to characterize how TNTs are molecularly and mechanistically differentiated from filopodia. We previously demonstrated that a filopodia-promoting Cdc42/IRSp53/VASP network negatively regulates TNT formation and impairs TNT-mediated intercellular vesicle transfer in neuronal cells. Conversely, elevation of Eps8, an actin regulatory protein that inhibits the extension of filopodia in neurons, increases TNT formation in our cell model. We hypothesize that a switch in common actin regulatory complexes is critical in driving the formation of either TNTs or filopodia. We are currently elucidating how Eps8, a key actin regulator that leads to actin filament stabilization in TNTs, may work synergistically with membrane deforming and membrane curvature sensitive proteins (inverted BAR (I-BAR) domain proteins such as IRSp53 and IRTKS which have nM affinities for Eps8) in the formation of TNTs.

During the student's internship, s/he will learn how to culture cells and to transfect them with expression vectors of the proteins of interest. In addition, the student will be trained to utilize both laser scanning and spinning disc confocal microscopes to acquire data on both fixed- and live-cell samples to characterize the protein cooperation in the mechanism of TNT formation and their functionality. Additionally, fluorescent recovery after photobleaching (FRAP) measurements will also be performed by the student to assess the development of actin within the TNTs. Consequently, interns will also gain computational expertise utilizing ImageJ/Fiji for image-based analysis. Finally, the student will be trained to use cell cytometry methods (e.g., Amnis ImageStream Mark II) in order to

evaluate the functionality of these TNTs by measuring the transfer of vesicles, or mitochondria, between donor and acceptor cell populations.

By the end of the internship, the student will gain not only new practical lab skills, but will also develop their independence in laboratory research, crucial for her/his growth as a young scientist. Additionally, by interacting with an internationally diverse team of PhDs and Postdocs, the student will have a unique opportunity to receive productive feedback on her/his work, develop public speaking skills within a friendly but professional environment, and to discuss opportunities/career paths in academic-based research after university.

Tutor/supervisor

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

1. Dilsizoglu Senol A, Pepe A, Grudina C, Sassoon N, Reiko U, Bousset L, Melki R, Piel J, Gugger M, Zurzolo C. **Effect of tolytoxin on tunneling nanotube formation and function,** Sci. Rep. 2019 9:5741.
2. Sartori-Rupp A, Cordero Cervantes D, Pepe A, Gousset K, Delage E, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, Zurzolo C. **Correlative cryo-electron microscopy reveals the structure of TNTs in neuronal cells,** Nat. Commun. 2019 10:342.
3. Zhu S, Bhat S, Syan S, Kuchitsu Y, Fukuda M, Zurzolo C. **Rab11a-Rab8a cascade regulates the formation of tunneling nanotubes through vesicle recycling,** J. Cell. Sci. 2018 131(19).
4. Loria F, Vargas JY, Bousset L, Syan S, Salles A, Melki R, Zurzolo C. **α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading,** Acta Neuropathol. 2017 134:789-808.
5. Delage E, Cervantes DC, Pénard E, Schmitt C, Syan S, Disanza A, Scita G, Zurzolo C. **Differential identity of Filopodia and Tunneling Nanotubes revealed by the opposite functions of actin regulatory complexes,** Sci. Rep. 2016 6:39632.
   Abounit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R, Zurzolo C. **Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes,** EMBO J. 2016 35:2120-2138.
6. Abounit S, Delage E, Zurzolo C, **Identification and Characterization of Tunneling Nanotubes for Intercellular Trafficking,** Curr. Protoc. Cell Biol. 2015 67:12.10.1-21.

7. Gousset K, Marzo L, Commere PH, Zurzolo C. **Myo10 is a key regulator of TNT formation in neuronal cells,** J. Cell. Sci. 2013 126:4424-35.
8. Costanzo M, Abounit S, Marzo L, Danckaert A, Chamoun Z, Roux P, Zurzolo C. **Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes,** J. Cell. Sci. 2013 126:3678-85.
9. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, Olivo-Marin JC, Männel D, Zurzolo C. **Prions hijack tunnelling nanotubes for intercellular spread,** Nat. Cell Biol. 2009 11:328-36.

Scientific or technical background required for work program

Previous experience in cell culture, cell and molecular biology techniques, and fluorescence microscopies is preferred but not mandaotry. We are seeking motivated candidates who share our passion for science, are enthusiastic about learning new techniques, and are interested in combining multdisciplinary approaches at the forefront of biological research.

Title of the work program 9

Role of stromal cells in immunity at barrier surfaces

Description of the work program

Our lab has previously shown that mesenchymal stromal populations have essential roles in inflammation (JI 2009), tissue repair (Nature Medicine 2012) and maintenance of the intestinal stem cell niche (PNAS 2017). In this project, we will investigate the role of stromal cells in the regulation of immune responses at barrier surfaces (such as skin, intestine). To that aim, we will analyze WT and mutant conditional knock-out mice for candidate molecules that have an essential role in the stromal crosstalk with immune cells. Using mouse models of injury/inflammation, we will measure the quality and intensity of the immune response in WT and mutant mice (by FACS), visualize the stromal crosstalk with immune cells (by confocal microscopy) and investigate the molecular mechanism underlying stroma-immune crosstalk (by transcriptomics). Altogether this project will allow us to determine the impact of stromal cells in immunity at barrier surfaces.

Tutor/supervisor

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

Di Carlo, S.E. & Peduto, L. The perivascular origin of pathological fibroblasts. *J Clin Invest* 128, 54-63 (2018)

Peduto L, Sansonetti PJ, Nigro G. Intestinal stem cells and their niche at homeostasis and under stress. Book chapter in **"Advances in Stem Cells and their niche"**. Elsevier, doi.org/10.1016 /bs.asn.2017.12.002 (2018)

Emgard J, Kammoun H, Garcia-Cassani B, Chesne J., Parigi SM, Jacob JM, Cheng HW, Evren E, Das S, Czarnewski P, Sleiers N, Melo-Gonzales F, Kvedaraite E, Svensson M., Scandella E, Hepworth MR, Huber S, Ludewig B, Peduto L, Villablanca EJ, Veiga-Fernandes H, Pereira JP, Flavell RA, Willinger T. Oxysterol sensing through GPR183 (EBI2) controls the lymphoid tissue-inducing function of innate lymphoid cells and colonic inflammation. **Immunity**, 48(1):120-132.e8 (2018)

Stzepourginski I, Nigro G, Jacob JM, Dulauroy S, Sansonetti PJ, Eberl G, Peduto L. CD34+ mesenchymal cells are a major component of the intestinal stem cells niche at homeostasis and after injury. **PNAS,** 114(4): E506-E513 (2017)

Dulauroy, S., Di Carlo, S.E., Langa, F., Eberl, G. & Peduto, L. Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nature Medicine* 18, 1262-1270 (2012)

Schilte C, Couderc T, Chretien F, Sourisseau M, Gangneux N, Guivel-Benhassine F, Kraxner A, Tschopp J, Higgs S, Michault A, Arenzana-Seisdedos F, Colonna M, Peduto L, Schwartz O, Lecuit M, and Albert ML. Type I IFN controls chikungunya virus via its action on non-hematopoietic cells. **J Exp Med**, 207 (2): 429-442 (2010)

Peduto L, Dulauroy S, Lochner M, Spaeth G, Morales MA, Cumano A, and Eberl G. Inflammation recapitulates the ontogeny of lymphoid stromal cells. **J Immunol**, 182 (9): 5789-5799 (2009)

Scientific or technical background required for work program

To achieve this project, the candidate will use different experimental approaches including multiparametric FACS after isolation from tissues, transcriptomics (qPCR arrays or RNAseq), and confocal microscopy to visualize the stromal crosstalk within the tissue microenvironment. Highly motivated and creative candidates with a strong interest in immunology /stromal biology are strongly encouraged to apply. Previous experience with FACS, transcriptomics or confocal microscopy would be a plus, but is not required.

Title of the work program 10

Characterization of the RNA degradosome of the bacterial pathogen *Helicobacter pylori*

Description of the work program

In our laboratory, we are studying the pathogen *Helicobacter pylori* (*Hp*). This bacterium chronically colonizes the stomach of half of the human population and causes gastritis that can evolve into gastro-duodenal ulcers, MALT lymphoma or gastric adenocarcinoma that leads to more than 800,000 deaths/year.

Post-transcriptional regulation is a major level of control of gene expression that relies on multi-protein complexes, some of which include ribonucleases and, in bacteria, are called RNA degradosomes, central for RNA processing and degradation. Our lab previously established that *Hp* possesses a minimal RNA-degradosome composed by the essential endo- and 5'-3' exoribonuclease RNase J and RhpA, the sole DEAD-box RNA helicase encoded in the genome of *Hp* [1,2]. This complex plays a major role in mRNA decay and can be found associated to ribosomes [1,3]. We are now interested in different aspects of the functioning of this molecular machine, such as its subcellular localization, other possible partners and some of its potential regulatory mechanisms. For these studies, we use imaging technologies as well as biochemical and genetic approaches both in *Hp* and in *E. coli*. The student will be involved in several aspects of this project.

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

[1]   Redko Y, Aubert S, Stachowicz A, Lenormand P, Namane A, Darfeuille F, Thibonnier M, De Reuse H (2013) A minimal bacterial RNase J-based degradosome is associated with translating ribosomes. *Nucleic Acids Res.* **41**, 288–301.

[2]   El Mortaji L, Aubert S, Galtier E, Schmitt C, Anger K, Redko Y, Quentin Y, De Reuse H (2018) The sole DEAD-box RNA helicase of the gastric pathogen Helicobacter pylori is essential for colonization. *MBio* **9**, e02071-17.

[3]   Redko Y, Galtier E, Arnion H, Darfeuille F, Sismeiro O, Coppée J-Y, Médigue C, Weiman M, Cruveiller S, De Reuse H (2016) RNase J depletion leads to massive changes in mRNA abundance in *Helicobacter pylori*. *RNA Biol.* **13**, 243–253.

Scientific or technical background required for work program

Training in microbiology, biochemistry, molecular biology or related areas is required, as well as a good level of English. Laboratory experience in molecular biology and microbiology is a plus.

Title of the work program 11

Elucidating the role of the m^{6}A epitranscriptome in the human malaria parasite *Plasmodium falciparum*

Description of the work program

Plasmodium falciparum causes the most severe form of malaria, a mosquito-borne infectious disease killing 400 thousand (mostly children) and infecting more than 200 million people each year. All symptoms of the disease are caused by the replication of the parasite within human red blood cells, a process that is controlled by a precisely timed cascade of gene expression. Yet, the mechanisms that regulate this pattern of gene expression are still not fully characterized. To this end, we recently performed a comprehensive analysis of mRNA modifications as a potential new mechanism for the post-transcriptional regulation of protein synthesis. This study identified methylation of adenosines (m^{6}A) as an extensive modification of the *P. falciparum* transcriptome at levels higher than in any other eukaryote investigated. We found that m^{6}A affects both the stability and translational efficiency of mRNA transcripts on a transcriptome-wide level, thereby adding m^{6}A as a crucial gene regulatory mechanism for the proliferation of the human malaria parasite inside it's human host. We now aim to understand how m^{6}A can dynamically modulate the fate of mRNAs by characterizing the proteins that specifically bind to m^{6}A-methylated transcripts. To do so, we are applying a wide range of techniques, including targeted gene disruption using CRISPR/Cas9, genome-wide sequencing approaches (e.g. RNA/DNA-seq), protein mass-spectrometry and life-cell imaging.
The project is well embedded in our broader effort in this emerging field of research and provides plenty of opportunities for the student to shape it based on her/his own interests. The student will enter in this highly interdisciplinary field of research by getting trained and applying a wide array of techniques both in parasitology and genetics, ranging from parasite cell and mosquito culture, state-of-the art techniques in molecular biology up to bioinformatic analysis of next-generation sequencing data.

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

Baumgarten, S. et al. Transcriptome-wide dynamics of extensive m^{6}A mRNA methylation during *Plasmodium falciparum* blood-stage development. ***Nature Microbiology*** (2019). doi:10.1038/s41564-019-0521-7

Zanghì, G. et al. A specific PfEMP1 Is expressed in *P. falciparum* sporozoites and plays a role in hepatocyte infection. ***Cell Reports*** 22, 2951–2963 (2018).

Bryant, J. M. et al. CRISPR/Cas9 genome editing reveals that the intron is not essential for *var2csa* gene activation or silencing in *Plasmodium falciparum*. ***mBio*** (2017). doi:10.1128/mBio.00729-17

Guizetti, J., Barcons-Simon, A. & Scherf, A. Trans-acting GC-rich non-coding RNA at *var* expression site modulates gene counting in malaria parasite. ***Nucleic Acids Research*** (2016). doi:10.1093/nar/gkw664

Scientific or technical background required for work program

The 'Biology of Host-Parasite Interactions (BIHP)' Unit at the Institut Pasteur, lead by Prof Artur Scherf, spearheaded the discovery of gene regulatory mechanisms over more than two decades now, with a main focus on the epigenetic control of gene regulation. To study those mechanisms, we routinely use a wide array of techniques in literally all stages of the parasites life cycle, including genome-wide approaches such chromatin-immunoprecipitation (ChIP-seq) and RNA/DNA sequencing as well as targeted genome editing using CRISPR/Cas9. The great expertise of studying *P. falciparum* molecular biology in our laboratory is backed up by state-of-the art equipment including free access to Illumina next-generation sequencer (NextSeq500) and it's accompanying equipment (e.g. a Agilent BioAnalyzer).

Experience in basic molecular biology (e.g. PCR, plasmid cloning) is definitely a plus for this project, but the student will also be accompanied throughout her/his time in our lab and will get trained on a wide array of more advanced techniques to successfully pursue her/his research.

Since we are a highly international lab with researchers from all over the world, our working language is English.

Apply now, we are looking forward to welcome you soon!

Title of the work program 12

Expanding the toolbox for the genetic manipulation in *Leptospira*

Description of the work program

Leptospira, including the agent of leptospirosis, belong to the phylum of Spirochetes, an evolutionarily and structurally unique group of bacteria. The basic biology and virulence factors of leptospires remain poorly characterized due to the paucity of genetic tools.

Numerous methods exist for fluorescently labeling proteins. In a previous study, the *gfp* allele has been transferred into *Leptospira* strains to produce fluorescent leptospires but the bacteria expressing the fluorescent alleles were not sufficiently bright to be clearly visible with conventional microscopes (Aviat et al., 2010). In a first project, we will test the use of FlAsH (Fluorescein Arsenical Helix binder) dye labeling for live imaging of *Leptospira* strains. This system has been used in other bacteria for intra- and extra-cellular protein labeling using biarsenical dyes that bind specifically to a short 6-amino acid tetracysteine motif. Using this technique, proteins could be viewed in live cells in real-time without the issues typically encountered with GFP (significant increase of protein size, proper protein folding, etc).

CRISPR/Cas9 technology has recently emerged as a powerful tool for genetic manipulation, including in fastidious organisms. We recently reported the complete gene silencing in the saprophyte *L. biflexa* when both dCas9 (a Cas9 nuclease mutant that retains DNA-binding activity thus preventing the binding of the RNA polymerase to promoter sequences) and single-guide RNA (sgRNA) targeting the coding strand of the gene of interest were expressed simultaneously (Fernandes et al. 2019). In a second project, we will generate new constructs and apply this technique to the pathogenic *Leptospira* spp. to specifically repress or modulate the expression of genes at any locus of interest.

This project will provide us with an opportunity to apply state-of-the-art approaches to the identification of gene function in *Leptospira* spp.

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

Aviat F, Slamti L, Cerqueira GM, Lourdault K, Picardeau M. Expanding the genetic toolbox for Leptospira species by generation of fluorescent bacteria. Appl Environ Microbiol. 2010 Dec;76(24):8135-42. doi: 10.1128/AEM.02199-10. Epub 2010 Oct 29.
Fernandes LGV, Guaman LP, Vasconcellos SA, Heinemann MB, Picardeau M, Nascimento ALTO. Gene silencing based on RNA-guided catalytically inactive Cas9 (dCas9): a new tool for genetic engineering in Leptospira. Sci Rep. 2019 Feb 12;9(1):1839. doi: 10.1038/s41598-018-37949-x.

Scientific or technical background required for work program

Experience with molecular biology techniques such as PCR, molecular cloning, etc

Title of the work program 13

Sea anemone as a new model to explore the molecular mechanisms in deafness

Description of the work program

The main goal of this project is to study the molecular functioning of the sensory cells of the Vertebrates inner ear, particularly those of the cochlea, the organ of hearing, using an original and alternative model: the sea anemone.

In vertebrates, the sensory cells of the inner ear have at their apex a hair bundle, immersed in a liquid and composed of actin filaments, the stereocilia.

These hair bundles play the role of antenna by detecting the liquid movements generated by the sound waves. During hair bundles deflections, mechanotransduction channels, located between the rows of stereocilia, will open and allow the massive entry of K^+ and Ca^{2+} ions inside the cells that will depolarize and release neurotransmitters at their base, which will transmit the auditory message to the auditory neurons and then to the brain.

The mechanotransduction channels are localized, on the stereocilia, at the base of small links, called tip-links, composed of two cadherins, the cadherin 23 (cdh23) and the protocadherin 15 (pcdh15). These two proteins belong to the family of Usher type I proteins.

There are two main groups of Usher proteins: the Usher proteins of type I: Myosin 7a (usher 1B), Harmonin (usher 1C), cadh23 (usher 1D), Pcdh15 (usher 1F) and Sans (usher 1G); and the Usher proteins of type II: Usherin (usher 2A), VLGR1 (usher2C) and Whirlin (usher 2D).

All these proteins play crucial roles in the development of inner ear sensory cells and the setting up of the hair bundles, as well as for their function. Their mutations all lead to Usher syndromes in humans, associating deafness of different degrees, associated with blindness and deficits in balance (Petit, 2001).

In order to study the roles of these proteins as well as their interactions in molecular networks, transgenic mouse models are currently available, in which the genes encoding Usher proteins have been invalidated. These models have led to considerable advances in understanding the cellular and molecular mechanisms of auditory function. However, they also present some inconvenience which can be limits for experimental studies. Thus, the mammalian cochlea has a very small number of sensory cells (a few thousand) and these cells cannot regenerate. It is therefore not easy, with this type of model, to dispose of cellular material in sufficient quantity to carry out, for example extractions of proteins, which would make it possible to find partners of the Usher proteins, and in particular the proteins composing the mechanotransduction channels and their molecular partners.

It would therefore be very interesting to develop a new model, with sensory cells that have mechano-sensitive structures similar to those of the sensory cells of the inner ear, with hair bundles and similar molecular components. And it would be necessary that this model has available sensory cells in large quantities, able to regenerate. This organism should also be easily breeded in the laboratory and it must be possible to perform genetic manipulation on this model in order to dissect the molecular mechanisms involved in mechano-detection.

Some years ago, one group of the University of Louisiana in Lafayette have shown that some cnidarians, the sea anemones, have sensory cells with the hair bundle-like structure with similar morphology to those of superior animals, and more particularly, they are similar to the vertebrate inner ears hair bundles (Watson and Mire, 1997 ; Watson and Mire, 1999).

Sea anemones are marine invertebrates and are among the simplest animals that use actin made hair bundles-like structures to detect vibrations in the weather environment . These mechano-receptive structures, located mainly all along their tentacles, allow them to detect their prey swimming around them. The prey are captured due to hair bundles stimulation, by a very specialized structure linked to hair bundles and localized inside the so called cnidocyte: the nematocyst.

Hair bundles-like structures of sea anemones seem to be truly closed ''ancestors'' of vertebrate hair bundles. This is then a very good and useful model for vertebrate hair bundles. Each tentacle of a sea anemone have many hair bundles and the tentacles can regenerate. This makes possible to easily explore the dynamics of the developing hair bundle structures.

One of the first steps of our project is thus to "validate" the model of sea anemone for the study of the molecular components of the hair bundles of the sensory cells of the inner ear.

We will use a small sea anemone : *Nematostella Vectensis*, wich is actually a very studied model and for which many well developped molecular experimental approaches are available. Within non-bilaterian animals, Nematostella has been the first whose genome was sequenced (Putnam et al, 2007) and this allowed to highlight a surprisingly conserved genetic structure, which shows that there is an unexpected complexity of the ancestral genome in bilaterians and cnidarians (Technau and Schwaiger, 2015 ; Sebé-Pedros et al, 2018). As a small sea anemone, it can be easily breed and stored in the laboratory and it is easy to induce gametogenesis and oviposition (Fritzenwanker and Technau, 2002). This makes it easy to use different molecular biology techniques, including CRISPR / Cas9 mediated genome editing to obtain a variety of transgenic lines.

Nematostella has therefore become a good model organism not only to carry out comparative studies, but also to address the study of general questions in biology.

We have observed the morphology of the hair bundles in Nematostella, using the scanning electron microscopy (SEM), and we could observe, on the surface of the tentacles, some structures with a morphology very similar to that of the hair bundles of the sensory cells of vertebrates inner ears.

In parallel, immunohistochemical experiments carried out on these tentacles of Nematostella, using antibodies designed against usher proteins, showed labellings in the hair bundles similar to those observed in vertebrates inner ears, confirming that Nematostella could really be a good model for studying the properties and interactions between Usher-like proteins.

In addition, the use of the database generated by our collaborator Eric Rottinger's team in IRCAN, Nice, France, allowed us to confirm that homologues of these Usher-like proteins are well expressed in Nematostella (Warmer et al, 2018). We have also observed that many other proteins that play crucial roles in the functioning of the vertebrate cochlear sensory cells are also expressed in Nematostella, and their genes have sequences for some that are highly conserved.

We would therefore be very interested in the possibility of hosting a student within the work frame of the European Erasmus + program, to assist us in this project, and so that he/she can train in different experimental approaches, associating molecular biology techniques (generation of animals where genes are invalidated by the CRISPR / Cas-9 technique), immunohistochemistry, electrophysiology and electron microscopy.

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

Selected Publication :

Michel V, Booth KT, Patni P, Cortese M, Azaiez H, Bahloul A, Kahrizi K, Labbé M, Emptoz A, Lelli A, Dégardin J, Dupont T, Aghaie A, Oficjalska-Pham D, Picaud S, Najmabadi H, Smith RJ, Bowl MR, Brown SD, Avan P, Petit C, El-Amraoui A. CIB2, defective in isolated deafness, is key for auditory hair cell mechanotransduction and survival. EMBO Mol Med. 2017 ;9(12):1711-1731. doi: 10.15252/emmm.201708087.

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Scientific or technical background required for work program

The candidate will be motivated to work on an exciting new marine model, and he/she should have knowledge of various molecular and cellular biology techniques such as western blot, cell culture, transfection, immunohistochemistry. He/she will also have knowledge of otpic imaging techniques (confocal microscopy) and electron microscopy imaging.

Title of the work program 14

Defining the nature of IL-17A-producing cells and the role of IL-23 in inflammatory disease

Description of the work program

Chronic inflammatory diseases (CID) such as spondyloarthritis (SpA) are a group of clinically heterogeneous, unrelated conditions that share common inflammatory pathways and derive from aberrant immune responses. Genome-wide association studies (GWAS) performed in several CID have highlighted disease associations with loci linked to molecular pathways not previously known to be involved in pathogenesis, suggesting new directions in the study of disease mechanisms[1].

GWAS data, together with mouse models of autoimmune disease, demonstrated that cells producing the pro-inflammatory cytokine IL-17 play a pivotal role in the initiation of inflammatory diseases[2]. The implication of the IL-23/IL-17 axis in SpA is supported by the finding that several of the non-MHC loci genetically linked with SpA are associated with genes in this pathway (*IL23R, IL12B, IL6R, IL1R2, RORC, RUNX3, TYK2, JAK2, CARD9*)[3,4]. IL-23 is important for the expansion and the functional activity of the Th17 cell subset[5]. However, several studies have suggested that IL-23 may also regulate the function of IL-17-producing innate immune cells, which express the IL-23R. Systemic expression of IL-23 in mice induces hallmarks of SpA, such as enthesitis and sacroiliitis, by acting on a population of CD3$^+$CD4$^-$CD8$^-$RORγt$^+$ entheseal resident lymphocytes expressing the IL-23R[6]. IL-23R-expressing γδ T cells are enriched in the peripheral blood of SpA patients[7] and IL-23 has been shown to increase production of IL-17 and IL-21 by RORγt$^+$ iNKT and γδ T cells[8].

Taken together, these data suggest that the inflammatory response in SpA may be the result of a complex interplay of different immune cell types and that the IL-23/IL-17 pathway may play a key role in the human disease. Treatment of SpA with IL-17A inhibitors has proven to be effective[9], however, a phase 2 study testing a recently developed IL-23 inhibitor did not show any clinically improvement compared to placebo in patients with active SpA[10]. The latter finding was unexpected because of the strong GWAS association of *IL23R* with SpA[1] and because IL-17A was shown to be downstream of IL-23 in murine CD4$^+$ T cells[11].

This project will build on our previous work addressing fundamental and translational aspects of lymphocyte biology in human CID[4,12], using spondyloarthritis (SpA) as a model[13].

Objectives

We will assess the function of IL-23 on innate and adaptive T cell populations, and investigate the nature of potential IL-23-independent, IL-17A-producing cells.

1. Defining the nature of IL-17A-producing cells in SpA

The very low frequency of IL-17A-producing cells and their observed plasticity have made a detailed molecular analysis challenging. To directly investigate all IL-17A-producing circulating leukocytes, we will isolate IL-17A-producing cells from whole blood of SpA patients, and will define their single-cell transcriptomes by "Cellular Indexing of Transcriptomes and Epitopes by sequencing" (CITE-seq)[14].

2. Investigate the role of IL-23 in SpA

Key evidence of the importance of IL-23 in autoimmune inflammation came from the analysis of mice deficient of *Il23a* or *Il23r*[5,15], or IL-23 overexpression in a mouse SpA model[6]. Less is known about the biologic function of IL-23 in human inflammatory disease, although GWAS results strongly suggest a role of this cytokine in several CID[1]. We have previously shown that stimulation of activated human CD4$^+$ T cells with IL-23 increased production of both IL-17A and IFNg[4], however, additional effects of IL-23 on human immune responses, in particular on "innate" T cell populations are poorly studied.

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

1. Coffre M, Roumier M, Rybczynska M, Sechet E, Law HK, Gossec L, Dougados M, Bianchi E, Rogge L (**2013**) Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. *Arthritis & Rheumatology* 65: 1510-21.

2. Blijdorp ICJ, Menegatti S, van Mens LJJ, van de Sande MGH, Chen S, Hreggvidsdottir HS, Noordenbos T, Latuhihin TE, Bernink JH, Spits H, Rogge L, Baeten DLP, Yeremenko NG (**2019**) Expansion of Interleukin-22- and Granulocyte-Macrophage Colony-Stimulating Factor-Expressing, but Not Interleukin-17A-Expressing, Group 3 Innate Lymphoid Cells in the Inflamed Joints of Patients with Spondyloarthritis. *Arthritis & Rheumatology* 71: 392-402

3. Menegatti S, Bianchi E, Rogge L (**2019**) Anti-TNF Therapy in Spondyloarthritis and Related Diseases, Impact on the Immune System and Prediction of Treatment Responses. *Frontiers in Immunology* 10: 382.

4. Bianchi E, Rogge L (**2019**) The IL-23/IL-17 pathway in human chronic inflammatory diseases-new insight from genetics and targeted therapies. *Genes & Immunity* 20: 415-425.

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5. Yahia-Cherbal H, Rybczynska M, Lovecchio D, Stephen T, Lescale C, Placek K, Larghero J, Rogge L, Bianchi E (**2019**) NFAT primes the human RORC locus for RORgt expression in CD4+ T cells. *Nature Communications*, accepted manuscript

Scientific or technical background required for work program

A solid knowledge of immunology is required to understand the basis of this project. Experience in cell culture and immunological methods will be very helpful.

Title of the work program 15

Optimization of TCR transfer vectors for HIV immunotherapy

Description of the work program

Rare cases of spontaneous control of HIV infection reveal that the human immune system has the capacity to mount an efficient antiviral response against HIV. Patients who contain HIV replication in the absence of therapy, called HIV Controllers, show signs of particularly efficient T cell responses, and maintain full CD4 helper function in the long term. We obtained recent evidence that CD4+ T cells of these rare patients preferentially express a particular set of shared T cell receptors (TCRs) directed at HIV capsid. These shared or "public" TCRs were found to detect Gag antigen with unusually high affinity. When transferred into healthy donor CD4+ T cells, the Gag-specific TCRs conferred properties characteristic of HIV Controller CD4+ T cell responses, including high antigen sensitivity and the capacity to produce multiple cytokines simultaneously. Of note, some of these TCRs were of sufficiently high affinity to confer cytotoxic capacity to both CD4+ and CD8+ T cells. Thus, transferring a single shared TCR proved sufficient to induce an efficient antiviral T cell response directed at HIV.

We propose to apply these findings to the field of HIV immunotherapy, by developing TCR transfer lentivectors that could be used to confer efficient anti-HIV T cell responses. The main objective of the project will be to define the TCR sequences that confer optimal T cell functions. The student will extend the range of Gag-specific TCRs tested, and will compare their properties to those of newly characterized Env-specific TCRs. The TCRs will be sequenced, inserted into lentivectors, and tested for cell surface expression by flow cytometry using TCR-specific antibodies and MHC II tetramers. The lentivectors will then be tested in T cells for their capacity to confer HIV-specific recognition, using intracellular cytokine staining. The cytotoxic function of TCR-transduced T cells will be analyzed by measuring the elimination of Gag- or Env-expressing target cells. The long-term goal will be to use the optimized TCR lentivectors in a humanized mouse model, to test their capacity at eliminating HIV-infected cells in vivo, and evaluate their potential as immunotherapeutic tools to restore efficient immune responses in HIV-infected patients.

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

- Vingert B., Perez-Patrigeon S., Jeannin P., Lambotte O., Boufassa F., Lemaître F., Kwok W.W., Theodorou I., Delfraissy J.F., Thèze J., and Chakrabarti L.A. ; for the ANRS EP36 HIV Controllers

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- Mukhopadhyay M, Galperin M, Patgaonkar M, Vasan S, Ho DD, Nouël A, Claireaux M., Benati D, Lambotte O, Huang Y, and Chakrabarti LA (2017, in press) DNA vaccination by electroporation amplifies broadly cross-restricted public TCR clonotypes shared with HIV controllers. **Journal of Immunology**

- Claireaux M., Galperin M., Benati D., Nouël A., Mukhopadhyay M., Klingler J., de Truchis P., Zucman D., Hendou S., Boufassa F., Moog C., Lambotte O., and Chakrabarti L.A. (2018). High frequency of HIV-specific circulating follicular helper T cells associates with preserved memory B cell responses in HIV controllers. **mBio** 2018 May 8;9(3). pii: 9:e00317-18.

- Galperin M.*, Farenc C.*, Mukhopadhyay M., Jayasinghe D., Decroos A., Benati D., Tan L.L., Ciacchi L., Reid H.H., Rossjohn J.**, Chakrabarti L.A.**, and Gras S.** (2018) CD4+ T cell mediated HLA class II cross-restriction in HIV controllers. **Science Immunology**. 2018 Jun 8;3(24). pii: eaat0687.

Scientific or technical background required for work program

We are looking for a highly motivated candidate with knowledge in the fields of Virology and/or Immunology, and good communication skills in spoken and written English. Prior expertise in viral vector engineering and flow cytometry will be a plus.

Title of the work program 16

RNA Helicase function in vertebrate sex-determination and human Disorders of Sex Development (DSD).

Description of the work program

The unit aims to understand the mechanism governing cell fate choice in the gonad and there are several key elements to the research strategy – one is using the latest technologies and cutting-edge approaches that will have a major impact on the research questions - such as genomic/exome sequencing that we have used since 2008 or more recently cellular reprogramming approaches that involve normal cells or cells from patients with well-defined mutations. These approaches use an important and unique inhouse resource, which is the biobank of material from sex-reversed individuals, related pathologies such as infertility and unique control individuals.

Sex determination (SD) is the process by which a sexually reproducing organism initiates differentiation as either male or female. Mechanisms of SD differ considerably among organisms despite their importance for sexual reproduction and the survival of species.[1] The mammalian gonad is unique in being bipotential. Emerging evidence suggests that commitment of a common progenitor to either male (Sertoli cell) or female (granulosa cell) fate is the outcome of a battle between mutually antagonistic male and female regulatory networks that canalize development down one pathway, whilst actively repressing the other.[1,2] What mechanism promotes cell fate choice is an important but poorly understood process in biology. In mammals, the function of *Sry* is to activate the expression of *Sox*9, which in turn induces Sertoli cell formation, the first testis somatic cell lineage.[1,2] *Sry* also initiates a positive feedback loop between *Sox*9 and *Fgf*9, which results in up-regulation of *Fgf*9 and repression of the ovarian gene *Wnt*4.[1,2] The DMRT gene family is involved in sexual development in birds, frogs, flies and worms. We established that, unlike mice, human *DMRT*1 is required for testis-determination.[3] The relationship between, and the components of, the SRY/SOX9 and DMRT1 networks are poorly understood. In mice, ovary formation requires both *Rspo*1/*Wn*t4/*β-catenin* and *Foxl*2 signaling networks; although the degree to which these networks interact is unknown.[2,3] The Rspo1/Wnt4 signaling network stabilizes β-catenin, which counteracts the establishment of a pro-testis Sox9/Fgf9 network.[2,3] *Foxl*2 is required to maintain the ovarian identity postnatally, since the absence of *Foxl*2 in the adult ovary leads to granulosa cells transdifferentiating into Sertoli cells.[3] The loss of *Dmrt*1 in adult Sertoli cells results in their trans-differentiation into granulosa and theca cells.[3] In both cases this is accompanied by widespread tissue reorganisation into either an ovary or testis highlighting the plasticity of adult gonad. In the past 30 years, research into vertebrate SD has focused on the testis SOX9/DMRT1 pathways and ovary RSPO1/WNT4/FOXL2 pathways. Due to the lack of conservation of SD in animals discoveries have been incremental and focused on understanding of these pathways. However the aetiology of majority of human individuals with sex-reversal are still unexplained suggesting that novel factors/pathways remain to be identified.[4]

In an exome screen of a cohort of individuals with 46,XY sex-reversal (SR, gonadal dysgenesis) or 46,XY testicular regression (lack of maintenance of testis) we identified 10 novel mutations in the putative DEAH-box RNA helicase *DHX*37.[5] All mutations are absent from public databases. Pathogenic *DHX*37 mutations are as frequent a cause of XY SR as mutations in the testis-determining gene *SRY*. One important and intriguing difference with the latter is that some patients show testicular regression, suggesting that DHX37 has roles in both gonad determination and maintenance. *DHX*37 is one the most conserved genes in the human genome and is intolerant to loss-of-function and missense variants.[6] Fisher`s Exact test (two-tailed) on the frequency of loss-of-function and missense variants observed in our DSD cases, with rare missense mutations from 32,500 control individuals of matched-ancestry (ExAC database) shows a significant enrichment in the DHX37 gene in the DSD cases (P value = 5.1x10-6). Evidence in favour of causality is more compelling when one considers the position of the mutations in the protein. With one exception the mutations are located in two conserved domains (RecA1: ATP binding DEAH-box Helicase, RecA2: C-terminal Helicase) with the amino acids involved conserved to yeast. All mutations are considered to be damaging by multiple predictive software.

Consistent with a role in SD, we show, DHX37 is expressed exclusively in the human Sertoli cell lineage at the moment of testis-determination. Although little data is available on the function of *DHX*37 in higher organisms, the yeast orthologue of *DHX*37, *Dhr*1 is required for ribosome biogenesis.[7,8,9] *Dhr*1 is a essential component of the SSU processome, where its activity drives the transition of the SSU processome to pre-40S and pre-60S maturation pathways.[10] A recent study has shown that human *DHX*37 has a similar role in ribosome biogenesis and interacts with equivalent protein and snoRNA components.[11] However, the exact function of DHX37 in human sex-determination is entirely unknown. We hypothesise that the RNA helicase DHX37 may have acquired a specific function in testis-determination involving RNA metabolism. We are approaching this problem using multiple approaches –

(i)   assay the impact of mutations on helicase activity.

(ii)   Identify biologically relevant RNA targets and protein-partners of DHX37 from human foetal Sertoli cells during testis-determination. We will use high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP). Specifically, we will use the enhanced (eCLIP) approach. This technique is reliant on good quality antibody and availability of appropriate tissue foetal tissue. Multiple commercial anti-DHX37 antibodies will be assayed and RNA targets of interest will be independently validated by RNA-immunoprecipitation (RIP) and qRT-PCR. Following optimization, protocols will be used on isolated Sertoli cells from the human (collaboration with Rod Mitchell, MRC Edinburgh), goat and pig testis. Similarly, protein-partners of DHX37 will also be defined from appropriate gonadal tissue by ChIP purification followed by LC-MS/MS.

(iii)   Model DHX37 mutations in a novel human ex-vivo model. We have established protocols to successfully derive Sertoli-like cells from human induced pluripotent cells (iPSCs (unpublished)). Briefly, iPSCs are subjected to directed differentiation using sequentially a conditioned medium containing defined concentrations of bFGF, BMP4 and retinoic acid and insulin transferrin selenium. The iPSCs derived from a normal male can differentiate to the mesoderm and intermediate mesoderm as indicated by the expression of stage appropriate markers (e.g. Brachaury and WT1). The

mesenchymal-to-epithelium transition and subsequent formation of Sertoli-like cells is characterised by the expression of the markers GATA4, NR5A1, SOX9, FGF9 and DMRT1. These cells do not express granulosa cell markers. Furthermore, these Sertoli-like cells have the ability to self-aggregate and form tubule-like structures. To understand the role of DHX37 in Sertoli cell development, we will use CRISPR/Cas9 approaches to introduce the pathogenic mutations in the REcA1 and RecA2 domains. The iPSCs will be subjected to directed differentiation into somatic lineages of the testis, using the protocols we already optimised. During the course of differentiation stage-specific markers will be used to identify the resultant lineages. Fetal/adult Sertoli cells will also be verified by detection of AMH and their ability to aggregate. The transcriptome of the Sertoli-like cell lines will be analyzed by RNA-seq and compared to controls. This data will indicate patient-specific atypical transcription networks as well as provide mechanistic insight into the stage specific deregulation of signalling, associated with DHX37 mutations during SD.

(iv)   Create mouse and zebrafish KI models of the Dhx37 mutants. This is done in collaboration with Serge Nef (Univ. Geneva). We do not know if DHX37 plays a similar role in testis-determination in these two species.

The successful candidate will be involved in identifying biologically relevant RNA targets and protein-partners of DHX37 as well as modeling and characterization of DHX37 mutations using human iPSC model (highlighted in bold).

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

5 selected from 2016-2019)

1: McElreavey K, Jorgensen A, Eozenou C, Merel T, Bignon-Topalovic J, Tan DS, Houzelstein D, Buonocore F, Warr N, Kay RGG, Peycelon M, Siffroi JP, Mazen I, Achermann JC, Shcherbak Y, Leger J, Sallai A, Carel JC, Martinerie L, Le Ru R, Conway GS, Mignot B, Van Maldergem L, Bertalan R, Globa E, Brauner R, Jauch R, Nef S, Greenfield A, Bashamboo A. Pathogenic variants in the DEAH-box RNA helicase DHX37 are a frequent cause of 46,XY gonadal dysgenesis and 46,XY testicular regression syndrome. Genet Med. 2019 Jul 24. doi: 10.1038/ s41436-019-0606-y.

2: Harris A, Siggers P, Corrochano S, Warr N, Sagar D, Grimes DT, Suzuki M, Burdine RD, Cong F, Koo BK, Clevers H, Stévant I, Nef S, Wells S, Brauner R, Ben Rhouma B, Belguith N, Eozenou C, Bignon-Topalovic J, Bashamboo A, McElreavey K, Greenfield A. ZNRF3 functions in mammalian sex determination by inhibiting canonical WNT signaling. Proc Natl Acad Sci U S A. 2018 May 22;115(21):5474-5479.

3: Bashamboo A, Eozenou C, Jorgensen A, Bignon-Topalovic J, Siffroi JP, Hyon C, Tar A, Nagy P, Sólyom J, Halász Z, Paye-Jaouen A, Lambert S, Rodriguez-Buritica D, Bertalan R, Martinerie L, Rajpert-De Meyts E, Achermann JC, McElreavey K. Loss of Function of the Nuclear Receptor NR2F2, Encoding COUP-TF2, Causes Testis Development and Cardiac Defects in 46,XX Children. Am J Hum Genet. 2018 Mar 1;102(3):487-493.

4: Bashamboo A, Eozenou C, Rojo S, McElreavey K. Anomalies in human sex determination provide unique insights into the complex genetic interactions of early gonad development. Clin Genet. 2017 Feb;91(2):143-156.

5: Bashamboo A, Don(ohoue PA, Vilain E, Rojo S, Calvel P, Seneviratne SN, Buonocore F, Barseghyan H, Bingham N, Rosenfeld JA, Mulukutla SN, Jain M, Burrage L, Dhar S, Balasubramanyam A, Lee B; Members of UDN, Dumargne MC, Eozenou C, Suntharalingham JP, de Silva K, Lin L, Bignon-Topalovic J, Poulat F, Lagos CF, McElreavey K, Achermann JC. A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. Hum Mol Genet. 2016 Aug 15;25(16):3446-3453.

Scientific or technical background required for work program

It is desirable for the candidate to have a life sciences degree with basic understanding of genetics. An experience of working with human stem cells (embryonic or adult) and expertise in bioinformatics will be an asset.

Title of the work program 17

Impacts of the mechanical forces of the gut on *E. histolytica* invasive process.

Description of the work program

Entamoeba histolytica, a pathogenic highly motile cell, is the etiological agent of amoebiasis. *E. histolytica* infection initiates by parasite adherence to the mucus, which is depleted and then the parasite binds to the epithelium provoking cell death and production of pro-inflammatory cytokines. We have shown using human colon explants that *Entamoeba* takes advantage of a dense collagen scaffold at the sub-epithelial level to migrate until the crypts of Lüberkhun and then penetrates the mucosa via the loose collagen meshwork causing a remodeling and the destruction of the extracellular matrix (ECM).

One of our aims is to know **whether there is a crosstalk between the intestine mechanical forces and the pathogen,** which could influence its adhesion, the formation of infection foci, the expression of virulent factors and finally the invasive process. To answer this question, we propose to **determine the impact of mechanical forces and the cell tension** (the mathematician and physician of the lab will do the modeling of the mechanical quantities) **on *E. histolytica* invasive process** using **organ-on-a-chip** technology.

- We will analyze and compare *Entamoeba* invasive process of a 2D monolayer of cells and on the gut-on-chip (3D structure).
- To monitor quantitatively the dynamics of *E. histolytica* intestinal invasion and determine the preferential entry and adhesion sites, we will acquire images with spinning disk and confocal microscopes, and will use fluorescent amoeba (cell tracker) and epithelial biological markers, on live and fixed samples.
- To distinguish what are the critical steps enhancing *E. histolytica* infections we will used deficient amoeba or purified factors known to be involved in the infectious process.

Tutor/supervisor

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

1-Labruyère E, Thibeaux R, Olivo-Marin JC, Guillén N. Crosstalk between *Entamoeba histolytica* and the human intestinal tract during amoebiasis. (2017). Review. Parasitology.
2. A. Boquet-Pujadas, T. Lecomte, M. Manich, R. Thibeaux, E. Labruyère, N. Guillén, J-C Olivo-Marin, and A. C. Dufour. BioFlow: a non-invasive, image-based method to measure speed, pressure and forces inside living cells. (2017). Nature Scientific report
3-Thibeaux R, Avé P, Bernier M, Morcelet M, Frileux P, Guillén N, Labruyère E. The parasite *Entamoeba histolytica* exploits the activities of human matrix metalloproteinases to invade colonic tissue. (2014). Nat Commun.

4-Thibeaux R, Dufour A, Roux P, Bernier M, Baglin AC, Frileux P, Olivo-Marin JC, Guillén N, Labruyère E. Newly visualized fibrillar collagen scaffolds dictate *Entamoeba histolytica* invasion route in the human colon. (2012). Cell Microbiol.

5- Dufour A, Thibeaux R, Labruyère E, Guillen N, Olivo JC. 3-D active meshes: fast discrete deformable models for cell tracking in 3-D time-lapse microscopy. (2011). IEEE Trans Image Process.

Scientific or technical background required for work program

- Cell biology or Biophysics or Microbiology .

Title of the work program 18

Impact of the microbiota on colonization resistance against *Listeria monocytogenes*

Description of the work program

The impact of microbiota on host physiology has emerged over the past two decades. Host-microbiota interaction is mutually beneficial: commensal bacteria are important for host metabolism and allow synthesis and adsorption of nutrients and in turn, the composition of the intestinal microbiota is influenced by environmental and host factors such as diet and host genetics. The intestinal microbiota is a highly rich and competitive environment which provides resistance to intestinal lumen colonization against bacterial pathogens, a process called colonization resistance. It thus constitutes a first line of defense against the model pathogen *Listeria monocytogenes* (*Lm*).

Lm is a Gram-positive bacterium which is responsible for listeriosis, one of the deadliest foodborne infections. *Lm* is a ubiquitous bacterium that thrives in various environments and can contaminate alimentary products. These properties favor *Lm* persistence and transmission. Intestinal colonization by *Lm* is mostly asymptomatic in humans, and the incidence of human listeriosis is low despite frequent and repeated exposures to *Lm*. Yet, in immunosuppressed individuals, the elderly and pregnant women, *Lm* can induce septicemia, central nervous system and maternal-fetal infections, respectively, which can be fatal (up to 30% overall mortality) or lead to severe complications.

This project aims at understanding the process of colonization resistance towards *Lm*, and investigate the role of microbiota composition in *Lm* persistence within the gut lumen. The Erasmus student will participate at all the stages of the development and the realization of this project.

First, using metagenomic data obtained from listeriosis patients compared to controls, the student will study whether and how specific microbial composition correlates with reduced colonization resistance towards *Lm*. Then, using a humanized mouse model of orally-acquired *Lm*, he/she will determine which components of the microbiota may restrict *Lm* colonization and potentially limit its invasiveness. The student will then modulate microbiota composition to assess functionally his/her results.

Tutor/supervisor

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

Maury MM, Bracq-Dieye H, Huang L, Vales G, Lavina M, Thouvenot P, Disson O, Leclercq A, Brisse S, Lecuit M. *Hypervirulent Listeria monocytogenes clones' adaption to mammalian gut accounts for their association with dairy products.* Nat Commun. 2019, 10(1):2488.

Disson O, Blériot C, Jacob JM, Serafini N, Dulauroy S, Jouvion G, Fevre C, Gessain G, Thouvenot P, Eberl G, Di Santo JP, Peduto L, Lecuit M. *Peyer's patch myeloid cells infection by Listeria signals through gp38^{+} stromal cells and locks intestinal villus invasion.* J Exp Med. 2018, 215(11):2936-2954.

Charlier C, Perrodeau É, Leclercq A, Cazenave B, Pilmis B, Henry B, Lopes A, Maury MM, Moura A, Goffinet F, Dieye HB, Thouvenot P, Ungeheuer MN, Tourdjman M, Goulet V, de Valk H, Lortholary O, Ravaud P, Lecuit M; MONALISA study group. *Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study.* Lancet Infect Dis. 2017, 17(5):510-519.

Moura A, Criscuolo A, Pouseele H, Maury MM, Leclercq A, Tarr C, Björkman JT, Dallman T, Reimer A, Enouf V, Larsonneur E, Carleton H, Bracq-Dieye H, Katz LS, Jones L, Touchon M, Tourdjman M, Walker M, Stroika S, Cantinelli T, Chenal-Francisque V, Kucerova Z, Rocha EP, Nadon C, Grant K, Nielsen EM, Pot B, Gerner-Smidt P, Lecuit M, Brisse S. *Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes.* Nat Microbiol. 2016, 2:16185.

Maury MM, Tsai YH, Charlier C, Touchon M, Chenal-Francisque V, Leclercq A, Criscuolo A, Gaultier C, Roussel S, Brisabois A, Disson O, Rocha EPC, Brisse S, Lecuit M. *Uncovering Listeria monocytogenes hypervirulence by harnessing its biodiversity.* Nat Genet. 2016, 48(3):308-313.

Disson O, Nikitas G, Grayo S, Dussurget O, Cossart P, Lecuit M. *Modeling human listeriosis in natural and genetically engineered animals*. Nat Protoc. 2009, 4(6):799-810.

Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. *A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier*. Science. 2001, 292(5522):1722-5.

Scientific or technical background required for work program

- Lab experience, including animal experiments if possible
- Interest in bioinformatics analysis of microbiota datasets
- Strong work ethics
- Scientific curiosity
- Ability to integrate in a new work environment and work in a team

Title of the work program 19

Left-right patterning of the heart

Description of the work program

The laboratory studies the mechanisms of heart morphogenesis, using the mouse as a model. In the last 5 years, we have developed several tools to image, simulate and quantify heart development and malformation in 3D. Our work in collaboration with clinicians of the Necker hospital for sick children is relevant to congenital heart defects.

The alignment of cardiac chambers is key for the correct plumbing of the blood, so that carbonated blood in the right heart is separated from oxygenated blood in the left heart (reviewed in Desgrange et al, 2018). We have recently reconstructed and modelled the initial process of the left/right asymmetric morphogenesis of the heart (Le Garrec et al., 2017). In this context, the research project aims at characterising the spatiotemporal dynamics of molecular asymmetries in embryonic cardiac cells, using transcriptomic approaches and 3D mapping of gene expression by High Resolution Episcopic Microscopy. The work is integrated within an ongoing PhD project.

Tutor/supervisor

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	https://www.researchgate.net/profile/Sigolene_Meilhac

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

2017 Nature Communications, 8:14582, C. Ragni*, N. Diguet*, J-F. Le Garrec, M. Novotova, T. Resende, Pop, N. Charon, L. Guillemot, L. Kitasato, C. Badouel, A. Dufour, J-C. Olivo-Marin, A. Trouvé, H. McNeill and S. Meilhac, Amotl1 mediates sequestration of the Hippo effector Yap1 downstream of Fat4 to restrict heart growth.

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

2018 Nature Reviews Cardiology 5(11):705-724, S. Meilhac and M. Buckingham, The deployment of cell lineages that form the mammalian heart

2018 Development 145(22):dev162776 doi10.1242, A. Desgrange, J-F. Le Garrec, and S. Meilhac, Left-right asymmetry in heart development and disease : forming the right loop

2019 Disease Models & Mechanisms, 12(7):dmm038356, A. Desgrange*, J. Lokmer*, C. Marchiol, L. Houyel and S. Meilhac, Standardised imaging pipeline for phenotyping mouse laterality defects and associated heart malformations, at multiple scales and multiple stages

Scientific or technical background required for work program

You have outstanding academic record in biology or medicine. You work with rigour and creativity. You have a positive and open-minded personality and enjoy team work. You have a strong interest in developmental biology.

Title of the work program 20

Epigenetic mechanisms underlying inter-individual phenotypic variation in response to pathogens

Description of the work program

It is well known that different individuals show different responses to pathogens, and a growing number of emerging approaches to prevent and treat human diseases are considering individual variation in genes and environment. However, the understanding of the molecular basis of this inter-individual variation is not fully elucidated. Importantly, inter-individual phenotypic variations are not only due to differences in genetic background. In fact, inter-individual phenotypic variation has also been observed in genetically-identical individuals, ranging from unicellular bacteria to multicellular organisms, even grown in constant environments. Therefore, it is becoming fundamental to study the contribution of non-genetic phenotypic variation in human disease. Because each individual in a human population has a different genetic and environmental background, it is difficult to address the contribution of epigenetic factors in inter-individual phenotypic variation among human populations. For this reason, in this project we will use isogenic populations of the nematode *C. elegans* as an animal host model system to study 1) how genetically-identical organisms show different responses to pathogens, 2) the epigenetic mechanisms underlying inter-individual phenotypic variation in response to pathogens, and 3) whether phenotypic variation can be epigenetically inherited across generations. Recent studies using populations of worms have shown how stress-induced small RNAs can be inherited for multiple generations and the capacity of worms to transmit pathogen avoidance phenotype across generation through small RNAs. Therefore, we will test whether epigenetic mechanisms, such as small RNAs, can contribute to the heritability of inter-individual phenotypic variations in response to bacterial infection. For this purpose, we will apply high-throughput genomic approaches using individual worms (single-worm RNA-seq) to detect transcriptional changes during pathogen infections across genetically-identical individuals. We will also create transcriptional fluorescent reporter strains to study inter-individual gene expression changes during pathogen infection using single-molecule approaches. These fluorescent reporters will be used as a sensor of pathogen-induced transcriptional changes in individual worms. Individual worms expressing high or low levels of fluorescent reporter will be evaluated for their ability to epigenetically propagate pathogen-induced gene expression changes across generations. Moreover, we will characterize these sorted animals for their ability to respond to pathogen. The sorting approach coupled with the fluorescent reporter will also allow us to biochemically characterize the role of small RNAs and other epigenetic factors in the inheritance of inter-individual phenotypic variation during pathogen infection.

This project is highly multidisciplinary and will allow the Master student to acquire diverse skills ranging from molecular biology (design and screening of transgenic C. elegans strains by CRISPR), biochemistry (immunoprecipitation and purification of protein complexes), developmental biology (phenotypic characterization of stress sensitive worms), imaging (monitoring transcriptional responses to pathogen in living C. elegans animals by microscopy and FACS sorting) and bioinformatics analysis of high-throughput genomic methods.

Tutor/supervisor

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

Cecere G., Hoersch S., O'Keeffe S., Sachidanandam R., Grishok A. *Global effects of the CSR-1 RNA interference pathway on transcriptional landscape*. **Nature Structural & Molecular Biology**, 2014 Apr;21(4):358-65.

Cecere G. and Grishok A. *A nuclear perspective on RNAi pathways in metazoans*. **Biochimica et Biophysica Acta - Gene Regulatory Mechanisms**, 2013 December 17.

Cecere G, Zheng GX, Mansisidor AR, Klymko KE, Grishok A. *Promoters recognized by forkhead proteins exist for individual 21U-RNAs.* Molecular Cell. 2012 Sep 14;47(5):734-45.

Scientific or technical background required for work program

We are looking for highly passionate and motivated science-driven candidates with good knowledge in Molecular Biology, Developmental and Computational biology. Candidates with experimental expertise in biochemistry, molecular biology, high-throughput sequencing technologies and/or bioinformatics analysis would be desired. Successful applicants are expected to work in an interdisciplinary team, managing multiple tasks, having good organizational skills.

Title of the work program 21

Design of a c-di-AMP reporter system in *Escherichia coli and in Streptococcus agalactiae.*

Description of the work program

Cyclic and dicyclic nucleotides play a key role in a great number of metabolic processes. The discovery of c-di-AMP is particularly attracting since it is essential in the model Gram positive bacterium *Bacillus subtilis*, but also in the human pathogens *Staphylococcus aureus*, *Streptococcus pneumoniae,* and *Listeria monocytogenes*. c-di-AMP is synthesized by a diadenylate cyclase from two ATP molecules and is degraded by a phosphodiesterase to yield pApA or AMP. Diadenylate cyclases are characterized by a conserved catalytic domain called DAC (Commichau *et al.,* 2015). Noticeably, Gram-negative bacteria do not synthesize c-di-AMP, (e.g. *Escherichia coli*) but c-di-GMP.

In *Mycobacterium smegmatis,* a c-di-AMP/transcription factor binding screen identified the first c-di-AMP receptor regulator: DarR. DarR specifically associates with c-di-AMP and negatively regulates the expression of its target genes. In fact, c-di-AMP stimulates the DNA binding activity of DarR to a 14bp palindromic sequence (*darO*) in a concentration-dependent manner. (Zhang *et al.,* 2013).

The overall objective of this project is to design a versatile reporter system based on *darR*, *darO* and *dac* to screen inhibitors of c-di-AMP synthesis in *E. coli* and to monitor the c-di-AMP level of different mutants of the gram-positive pathogen *Streptococcus agalactiae* available in the laboratory.

In 2013, the Goettingen IGEM Team described a c-di-AMP reporter system in *E. coli* where *darR* is placed under the control of a strong promoter and the *darR* operator *darO* upstream the GFP.
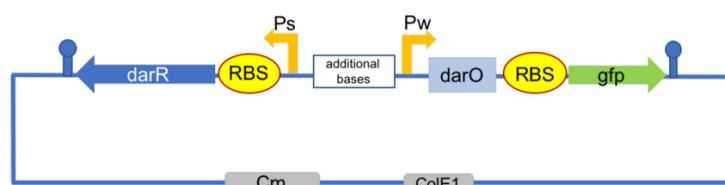

However, in this system, the binding of DarR to *darO*, in the absence of c-di-AMP, was strong enough to repress *gfp* expression.

This repression might be explained by an unbalanced expression of DarR and GFP. Thus, we propose to design regulatory elements (ie weak and strong promoters as well as efficient ribosome binding sites) to precisely control the expression of *darR* and that of *gfp*. Another solution would be the engineering of a DarR-*darO* pair displaying a lower affinity for each other by site-directed mutagenesis of critical DarR aminoacids or *darO* nucleotides identified *in silico*. A first series of mutations of the darO operator was tested and, as shown below, one of them allows the expression of gfp and is still repressed (although not completely) by darR in the presence of c-di-AMP.

- c-di-AMP

+ c-di-AMP
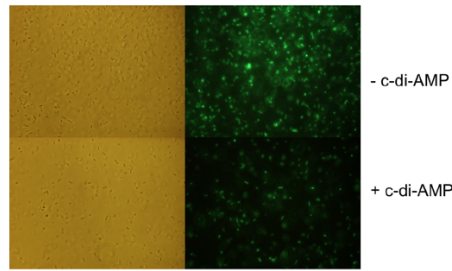
The functionality of the construct will be tested in an *E. coli* strain expressing an inducible form diadenylate cyclase from *S. agalactiae* (already available in the laboratory). In this system, uninduced and induced *E. coli* cells should be fluorescent and non-fluorescent, respectively. Once established, this c-di-AMP reporter system will be used to characterize mutants of the diadenylate cyclase in order to decipher the main catalytic properties of this enzyme.
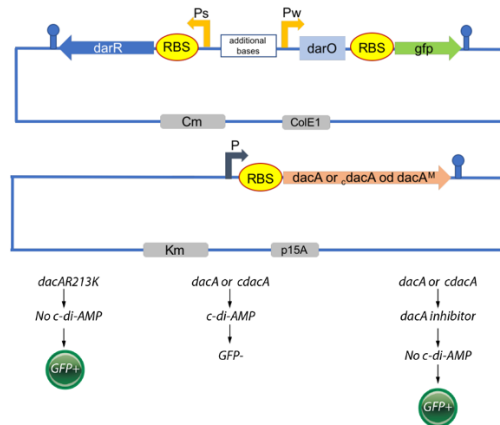
Whereas early work focused on the essentiality of c-diAMP, an increasing number of recent studies have reported the toxicity of c-di-AMP accumulation. All pathogenic species are diminished for virulence at high c-di-AMP levels. Thus targeting the c-di-AMP phosphodiesterase
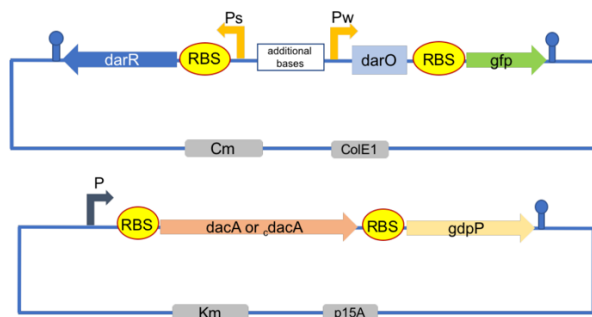
While this *E. coli* reporter system could be used to screen chemical libraries, the use of a Gram positive bacteria as a host is more pertinent as diadenylate cyclase(s) is(are) mainly absent from Gram negative bacteria and because their cell wall compositions differ.

The Gram positive bacteria chosen is *Streptococcus agalactiae*, 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.

S. agalactiae has only one diadenylate cyclase (DacA) and no equivalent of DarR that would compete with the *M. smegmatis* DarR carried by the plasmid pSC1B3 (see figure). In addition, a mutant deleted from the *dacA* gene can grow in minimal medium under anaerobiosis. These characteristics allow the screening of compounds targeting the diadenylate cyclase by monitoring the fluorescence. Positive hits will then be tested as growth inhibitors in complex medium.

In *S. agalactiae* also, the optimum design of regulatory elements will be a prerequisite. Remarkably, libraries of promoters and Ribosome Binding Sites (RBS) enabling the tuning of GFP concentration over five orders of magnitude, from 0.05 to 700 µM (Guiziou *et al.,* 2016) are available at the Bacillus Genetic Stock Center and thus could be tested as such in *S. agalactiae.* A problem associated with the use of GFP in anaerobic bacteria is the requirement of oxygen for posttranslational folding of the GFP to generate the fluorophore (Cubitt *et al.,* 1995). In addition, some of the GFP can also precipitate into weakly fluorescent inclusion bodies (Cormack *et al.,* 1996). Up to seven variants of GFP have been described and compared for live cell imaging in three low-GC-rich Gram-positive model organisms *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Lactococcus lactis* (Overkamp *et al.,* 2013). However, which of these variants is best suited for a certain bacterial strain, goal, or experimental condition is not clear and thus should be experimentally determined.

Once established, the *S.agalactiae* c-di-AMP reporter system will be introduced into our shuttle vector pTCV (Poyart and Trieu-Cuot, 1997) and used to monitor the c-di-AMP level of the different mutants available in the laboratory and also to screen chemical libraries to characterize DAC inhibitors as leads for the development of antibiotics active against *S. agalactiae.* Adaptation of this c-di-AMP reporter system in other Gram positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* will be considered.

Aymanns S, Mauerer S, van Zandbergen G, Wolz C, Spellerberg B. High-level fluorescence labeling of gram-positive pathogens. PLoS One. 2011;6(6):e19822. doi: 10.1371/journal.pone.0019822

Commichau FM, Dickmanns A, Gundlach J, Ficner R, Stülke J. A jack of all trades: the multiple roles of the unique essential second messenger cyclic di-AMP. Mol Microbiol. 2015, 97(2):189-204

Poyart, C., and P. Trieu-Cuot. A broad-host-range mobilizable shuttle vector for the construction of transcriptional fusions to beta-galactosidase in gram-positive bacteria. FEMS Microbiol Lett 1997, **156:**193-198.

Zhang L, Li W and He ZG. DarR, a TetR-like transcriptional factor, is a cyclic di-AMP-responsive repressor in *Mycobacterium smegmatis*. *J Biol Chem* 2013, 288:3085–3096.

Guiziou S, Sauveplane V, Chang HJ, Clerté C, Declerck N, Jules M and Bonnet J. A part toolbox to tune genetic expression in *Bacillus subtilis*. Nucleic Acids Res. 2016 44(15):7495-508.

Cubitt A G, Heim R, Adams S R, Boyd A E, Gross L A, Tsien R Y. Understanding, improving and using green fluorescent proteins. Trends Biochem Sci. 1995;20:448–455.

Cormack B P, Valdivia R H, Falkow S. FACS-optimized mutants of the green fluorescent protein (GFP) Gene. 1996;173:33–38.

Overkamp W, Beilharz K, Detert Oude Weme R, Solopova A, Karsens H, Kovács ÁT, Kok J, Kuipers OP, Veening J-W. 2013. Benchmarking various green fluorescent protein variants in *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Lactococcus lactis* for live cell imaging. Appl Environ Microbiol 79:6481–6490.

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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. eCollection 2018 Apr. PubMed PMID: 29659565; PubMed Central 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. Review. PubMed 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. PubMed PMID:

27414497; PubMed Central 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. doi: 10.1074/jbc.M113.545632. Epub 2014 Jan 15. PubMed PMID: 24429288; PubMed Central PMCID: PMC3937624.

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.

Flow-cytometer measurements and analysis

Title of the work program 22

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

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.

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.

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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 23

Immunometabolic host-pathogen interactions

Description of the work program

Infectious diseases are a major threat to human health and predictions suggest that over the next few decades, these will account for one of five deaths globally. In times where emergence of antibiotic-resistance in bacteria is occurring worldwide, understanding how bacteria cause disease is essential to finding new therapeutic approaches to tackle infection. Recent reports revealed intriguing functional connections between cellular metabolism and pathogen defenses in human cells, called immunometabolism. As intracellular bacteria are pathogens that survive and replicate inside human cells, the control of cellular metabolism and, specifically, mitochondrial functions by pathogens are key to establish infection. By using advanced automated microscopy, the selected candidate will monitor multiple cellular immunometabolic phenotypes in infected human cells in order to reveal specific metabolic interactions between human macrophages and two species of pathogenic intracellular bacteria, *Legionella pneumophila* and *Salmonella enterica*.

Tutor/supervisor

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

-Escoll P, Song OR, Viana F, Steiner B, Lagache T, Olivo-Marin JC, Impens F, Brodin P, Hilbi H, Buchrieser C. Legionella pneumophila Modulates Mitochondrial Dynamics to Trigger Metabolic Repurposing of Infected Macrophages. Cell Host Microbe. 2017;22(3):302-316.

-Escoll P, Buchrieser C. Metabolic reprogramming: an innate cellular defence mechanism against intracellular bacteria? Curr Opin Immunol. 2019 Jun 24;60:117-123.

-Song OR, Brodin P, Buchrieser C, Escoll P. Mitochondrial Dynamics and Activity in Legionella-Infected Cells. Methods Mol Biol. 2019;1921:205-220.

-Escoll P, Buchrieser C. Metabolic reprogramming of host cells upon bacterial infection: Why shift to a Warburg-like metabolism? FEBS J. 2018;285(12):2146-2160.

-Escoll P, Platon L, Buchrieser C. Roles of Mitochondrial Respiratory Complexes during Infection. Immunometabolism. 2019;1:e190011.

Scientific or technical background required for work program

Motivated scientific interest on the study of host-pathogen interactions
Cell culture of mammalian cells would be a highly appreciated technical skill

Title of the work program 24

Characterization of macrophage-directed, epigenetic inhibitors with activity on *Leishmania* intramacrophagic development

Description of the work program

All anti-leishmanial treatments suffer from the rise of drug resistant parasites. The dependence of *Leishmania* survival on subversion of the metabolic and immunologic functions of its macrophage host cell opens exciting possibilities for host-directed therapies that limit the emergence of drug resistance. Conducting phenotypic screening campaigns with 475 epigenetic inhibitors on dermotropic *L. amazonensis* and viscerotropic *L. donovani*, we identified compounds that target all major classes of DNA and histone modifying enzymes and affect positively or negatively intracellular parasite growth at 10 µM – 1 µM. On one hand, 27 of anti-leishmanial hits showed no activity against ex vivo, hamster-isolated amastigotes and extracellular promastigotes, revealing their host cell-directed mode of action. On the other hand, 50 compounds demonstrated a pro-parasite activity increasing substantially the growth of intramacrophagic amastigotes. The project proposed for the Erasmus student is to further characterize the mode of action of these pro-parasite compounds. The work program includes specific training for handling of primary macrophages and *Leishmania* developmental stages in a biosafety level 3 environment. Pharmacological experiments with selected epigenetic inhibitors will be carried out in parallel on *Leishmania*-infected macrophages and in host-cell free parasite stages to select eventually host-directed compounds that do not show activity on free parasites per se. Completion of this work program will allow the selection of a few host-directed epigenetic inhibitors for which systems level approaches (RNAseq and ChIPseq of infected, inhibitor treated cells) will be used with the aim to identify the macrophage mechanisms underlying increased intracellular parasite growth. A better understanding of these pathways and their epigenetic and transcriptional regulators may represent novel targets for host-directed therapy.

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

1. Rao SPS, Barrett MP, Dranoff G, Faraday CJ, Gimpelewicz CR, Hailu A, Jones CL, Kelly JM, Lazdins-Helds JK, Maser P, Mengel J, Mottram JC, Mowbray CE, Sacks DL, Scott P, **Spath GF**, Tarleton RL, Spector JM, Diagana TT. Drug Discovery for Kinetoplastid Diseases: Future Directions. ACS Infect Dis. 2019;5(2):152-7. PMID: 30543391.
2. **Lamotte S**, Aulner N, **Spath GF**, **Prina E**. Discovery of novel hit compounds with broad activity against visceral and cutaneous Leishmania species by comparative phenotypic screening. Sci Rep. 2019;9(1):438. PMID: 30679614.
3. **Piel L**, **Pescher P**, **Spath GF**. Reverse Epidemiology: An Experimental Framework to Drive Leishmania Biomarker Discovery in situ by Functional Genetic Screening Using Relevant Animal Models. Front Cell Infect Microbiol. 2018;8:325. PMID: 30283743.
4. **Bussotti G**, Gouzelou E, Cortes Boite M, Kherachi I, Harrat Z, Eddaikra N, Mottram JC, Antoniou M, Christodoulou V, Bali A, Guerfali FZ, Laouini D, Mukhtar M, Dumetz F, Dujardin JC, Smirlis D, Lechat P, **Pescher P**, El Hamouchi A, Lemrani M, Chicharro C, Llanes-Acevedo IP, Botana L, Cruz I, Moreno J, Jeddi F, Aoun K, Bouratbine A, Cupolillo E, **Spath GF**. *Leishmania* Genome Dynamics during Environmental Adaptation Reveal Strain-Specific Differences in Gene Copy Number Variation, Karyotype Instability, and Telomeric Amplification. mBio. 2018;9(6). PMID: 30401775.

5. Prieto Barja P, **Pescher P**, **Bussotti G**, Dumetz F, Imamura H, Kedra D, Domagalska M, Chaumeau V, Himmelbauer H, Pages M, Sterkers Y, Dujardin JC, Notredame C, **Spath GF**. Haplotype selection as an adaptive mechanism in the protozoan pathogen *Leishmania donovani*. Nature ecology & evolution. 2017;1(12):1961-9. PMID: 29109466.

6. **Lamotte S**, **Spath GF**, **Rachidi N**, **Prina E**. The enemy within: Targeting host-parasite interaction for antileishmanial drug discovery. PLoS Negl Trop Dis. 2017;11(6):e0005480. PMID: 28594938.

7. Dumetz F, Imamura H, Sanders M, Seblova V, Myskova J, **Pescher P**, Vanaerschot M, Meehan CJ, Cuypers B, De Muylder G, **Spath GF**, **Bussotti G**, Vermeesch JR, Berriman M, Cotton JA, Volf P, Dujardin JC, Domagalska MA. Modulation of Aneuploidy in *Leishmania donovani* during Adaptation to Different In Vitro and In Vivo Environments and Its Impact on Gene Expression. mBio. 2017;8(3). PMID: 28536289.

8. Bendjeddou LZ, Loaec N, Villiers B, **Prina E**, **Spath GF**, Galons H, Meijer L, Oumata N. Exploration of the imidazo[1,2-b]pyridazine scaffold as a protein kinase inhibitor. Eur J Med Chem. 2017;125:696-709. PMID: 27721154.

9. Durieu E, **Prina E**, Leclercq O, Oumata N, Gaboriaud-Kolar N, Vougogiannopoulou K, Aulner N, Defontaine A, No JH, Ruchaud S, Skaltsounis AL, Galons H, **Spath GF**, Meijer L, **Rachidi N**. From Drug Screening to Target Deconvolution: a Target-Based Drug Discovery Pipeline Using *Leishmania* Casein Kinase 1 Isoform 2 To Identify Compounds with Antileishmanial Activity. Antimicrob Agents Chemother. 2016;60(5):2822-33. PMID: 26902771.

10. Forestier CL, Spath GF, **Prina E**, Dasari S. Simultaneous multi-parametric analysis of *Leishmania* and of its hosting mammal cells: A high content imaging-based method enabling sound drug discovery process. Microb Pathog. 2014. PMID: 25448129.

11. Aulner N, Danckaert A, Rouault-Hardoin E, Desrivot J, Helynck O, Commere PH, Munier-Lehmann H, **Spath GF**, Shorte SL, Milon G, **Prina E**. High content analysis of primary macrophages hosting proliferating *Leishmania* amastigotes: application to anti-leishmanial drug discovery. PLoS Negl Trop Dis. 2013;7(4):e2154. PMID: 23593521.

12. Giraud E, **Lecoeur H**, Soubigou G, Coppee JY, Milon G, **Prina E**, Lang T. Distinct transcriptional signatures of bone marrow-derived C57BL/6 and DBA/2 dendritic leucocytes hosting live Leishmania amazonensis amastigotes. PLoS Negl Trop Dis. 2012;6(12):e1980. PMID: 23272268.

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14. **Lecoeur H**, de La Llave E, Osorio YFJ, Goyard S, Kiefer-Biasizzo H, Balazuc AM, Milon G, **Prina E**, Lang T. Sorting of *Leishmania*-bearing dendritic cells reveals subtle parasite-induced modulation of host-cell gene expression. Microbes Infect. 2010;12(1):46-54. PMID: 19786115.

15. Osorio y Fortea J, de La Llave E, Regnault B, Coppee JY, Milon G, Lang T, **Prina E**. Transcriptional signatures of BALB/c mouse macrophages housing multiplying *Leishmania amazonensis* amastigotes. BMC Genomics. 2009;10:119. PMID: 19302708.

Scientific or technical background required for work program

The student should have a foundational background in cell biology, basic concepts about infectious diseases and high interest in parasite host interactions.

Title of the work program 25

Investigate the mechanisms leading to the excretion of LdCK1.2 in the host cell via exosomes.

Description of the work program

A common feature of the most successful intracellular pathogens is the efficient manipulation of their host cell; this is true for bacteria such as *Mycobacterium* as well as for parasites. The manipulation of the host occurs mostly by the export of parasitic proteins into the host cell. *Mycobacterium, Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania* spp export proteins mainly via vesicles. In *Mycobacterium* and *Leishmania*, which invade macrophages, the export of proteins has been shown to modulate the immune response of the host cell. In our Unit, we are using *Leishmania* as a model to study these processes. Casein kinase 1 paralog 2 (*Ld*CK1.2) has been shown to be essential for intracellular parasite survival and released either as free protein or through exosomes as judged by proteomic studies. Moreover, several evidences suggest that *Ld*CK1.2 has been evolutionary selected for its capacity to interact with and phosphorylate host proteins to modulate macrophage biological processes. Indeed, *Ld*CK1.2 was shown to phosphorylate the human IFNAR1 receptor, which leads to the attenuation of the cellular response to interferon α/β. Thus the excretion of LdCK1.2 seems crucial for parasite survival in the host cell, but only little is known about the mechanisms regulating this process. There is at least one mechanism described to load cargo proteins into exosomes in mammalian cells: endocytosis of membrane proteins. Indeed, the cargos of the vesicles generated at the plasma membrane are delivered to early endosomes, which mature to become late endosomes and then multi-vesicular bodies (MVB). These MVBs can fuse with the plasma membrane to release the exosomes. AP2 complex is involved in the transport of cargos from the plasma membrane to the early endosomes through endocytosis by binding to clathrin-coated vesicles. Several evidences suggest that the AP2 complex could be implicated in the loading of LdCK1.2 into exosomes. We have previously shown that (i) LdCK1.2 binds to AP2 complex, (ii) LdCK1.2 phosphorylates the β2-adaptin subunit, and (iii) deletion of the AP2 subunits leads to lethality or severe growth and endocytosis defects. We are currently studying three of the four proteins that constitute the AP2 complex, the aim of this project will be to characterise further the role of the AP2 complex in LdCK1.2 trafficking by tagging the last AP2 subunit and deleting the corresponding gene using CRISPR/Cas9. Briefly, the intern will determine its localisation using confocal microscopy, its regulation using flow cytometry. He will perform kinase assays to determine whether the subunit is a substrate of LdCK1.2 and use FM4-64 or Concanavaline A to determine, by microscopy and flow cytometer, the ability of the mutant parasites to perform endocytosis. These experiments will allow us to have a better knowledge of the AP2 complex and its role in *Leishmania*.

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

1. A.L. Foucher, **N. Rachidi**, S. Gharbi, T. Blisnick, P. Bastin, I. K. Pemberton, and G.F. Späth. 2013. Apoptotic marker expression in the absence of cell death in staurosporine treated *Leishmania donovani*. Antimicrob Agents Chemother. 57 (3): 1252-61.
2. **N. Rachidi**, J.F. Taly, E. Durieu, O. Leclercq, N. Aulner, E. Prina, P. Pescher, C. Notredame, L. Meijer and G.F. Späth. 2014. Pharmacological assessment defines the *Leishmania donovani* casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection. Antimicrob Agents Chemother, 58 (3) 1501-1515. Corresponding author.
3. **N. Rachidi***, M. Cayla*, D. Schmidt-Arras, H. Rosenqvist, M. Wiese, and G. F. Späth. 2014. Transgenic analysis of the *Leishmania* MAP kinase MPK10 reveals an auto-inhibitory mechanism crucial for stage-regulated activity and parasite viability. PLOS Pathogens, 10(9), Online. *co-first authors*.
4. Gerald F. Späth, Sima Drini, **Najma Rachidi**. A touch of Zen: Post-translational regulation of the *Leishmania* stress response. 2015. Cellular microbiology 17 (5), 632-638. Review.
5. Durieu E, Prina E, Leclercq O, Oumata N, Gaboriaud-Kolar N, Vougogiannopoulou K, Aulner N, Defontaine A, No JH, Ruchaud S, Skaltsounis AL, Galons H, Späth GF, Meijer L, **Rachidi N**. From drug screening to target deconvolution: A target-based drug discovery pipeline using Leishmania casein kinase 1 isoform 2 to identify compounds with anti-leishmanial activity. 2016. *Antimicrob Agents Chemother* 60, 2822-2833; published online EpubMay (10.1128/AAC.00021-16).
6. Lamotte S, Späth GF, **Rachidi N** and Prina E. The enemy within: Targeting host-parasite interaction for antileishmanial drug discovery. 2017. PLoS Negl Trop Dis. Jun 8; 11(6): e0005480. Review.
7. Daniel Martel, Tom Beneke, Eva Gluenz, Gerald Frank Späth and **Najma Rachidi**. Characterisation of Casein kinase 1.1 in *Leishmania donovani* using the CRISPR Cas 9 toolkit. 2017. BioMed Research International, Article ID 4635605, 11 pages.
8. Bohm, T., Meng, Z., Haas, P., Henne-Bruns, D., **Rachidi, N**., Knippschild, U., and Bischof, J. (2019) The kinase domain of CK1delta can be phosphorylated by Chk1. *Bioscience, biotechnology, and biochemistry*, 1-13. *
9. Bryant, J. M., Baumgarten, S., Glover, L., Hutchinson, S., and **Rachidi, N**. (2019) CRISPR in Parasitology: Not Exactly Cut and Dried! *Trends in parasitology* (Review).
10. Hombach-Barrigah, A., Bartsch, K., Smirlis, D., Rosenqvist, H., MacDonald, A., Dingli, F., Loew, D., Spath, G. F., **Rachidi, N**., Wiese, M., and Clos, J. (2019) Leishmania donovani 90 kD Heat Shock Protein - Impact of Phosphosites on Parasite Fitness, Infectivity and Casein Kinase Affinity. *Scientific reports* 9, 5074. *
11. Xu, P., Ianes, C., Gartner, F., Liu, C., Burster, T., Bakulev, V., **Rachidi, N**., Knippschild, U., and Bischof, J. (2019) Structure, regulation, and (patho-)physiological functions of the stress-induced protein kinase CK1 delta (CSNK1D). *Gene* 715, 144005 (Review).*

Scientific or technical background required for work program

The student should have knowledge in cell biology. Basic technical skills in molecular biology will be a bonus.

Title of the work program 26

***Methanobrevibacter smithii* as model for cell growth and division in Archaea**

Description of the work program

Archaea are a neglected component of the human microbiome, where their role in health and disease, as well as their interaction with the host, are largely understudied. Even less is known about the molecular mechanisms that direct archaeal cell division and growth. Interestingly, while in most archaea the cell wall is formed by a proteinaceous surface (S-) layer, in Methanobacteriales and Methanopyrales it consists of pseudo-peptidoglycan (or pseudo-PG). The aim of this project is to investigate cell growth and division in archaea with a pseudo-PG cell wall by using the Methanobacteriales *Methanobrevibacter smithii*, the most abundant archaeal species in the human intestine, as a model. The genome of *M. smithii* contains homologues of the two cytoskeletal proteins MreB and FtsZ which are responsible in bacteria for the coordination of PG synthesis during cell elongation and division, respectively. Our preliminary data based on immunolabeling indicate that FtsZ forms a constricting ring at the division plane, as in bacteria. However, the precise role of FtsZ and MreB in cell growth and division in these archaea remains largely unknown.

Methods: We will cultivate *M. smithii* under anaerobic conditions in serum bottles. *M. smithii* cells will be incubated with the FtsZ stabilizing drug PC190723 and the two MreB inhibition drugs A22 and MP265. Additionally, treated and untreated cells will be immunolabelled with an anti-*M.smithii*-FtsZ antibody in combination with the cell wall stain WGA. To see the effect of the three drugs on cell shape and division, several hundreds of cells will be imaged by epifluorescence microscopy and their cell shape together with the fluorescent signal will be measured. Morphometric data will be used to construct fluorescent demographs to visualize the cellular localization of these proteins during the cell cycle.

Expected results: By using the FtsZ stabilizing drug we hope to block cell division in *M. smithii* cells and see a disturbance of the FtsZ localization pattern. If A22 and/or MP265 affect both MreB localization and cell growth/shape, this will indicate that MreB is the scaffold for pseudomurein incorporation in *M. smithii*. Those results will help to link for the first time the cytoskeletal proteins MreB and FtsZ to pseudo-PG synthesis during cell growth and division.

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

- Adam PS, Borrel G, Brochier-Armanet C, **Gribaldo S** (2017) The growing tree of Archaea: new perspectives on their diversity, evolution and ecology. ISME J 11(11): 2407-2425.

- **Pende N**, Wang J, Weber PM, Verheul J, Kuru E, Rittmann SKR, Leisch N, VanNieuwenhze MS, Brun YV, den Blaauwen T and Bulgheresi S. Host-Polarized Cell Growth in Animal Symbionts. *Current Biology 28(7):1039-1051.e5. doi: 10.1016/j.cub.2018.02.028*

- Leisch N, **Pende N**, Weber PM, Gruber-Vodicka HR, Verheul J, Vischer NOE, Abby SS, Geier B, den Blaauwen T and Bulgheresi S. Asynchronous division by non-ring FtsZ in the gammaproteobacterial symbiont of Robbea hypermnestra. *Nature Microbiology 2:16182. doi: 10.1038/nmicrobiol.2016.182*

- **Pende N**, Leisch N, Gruber-Vodicka HR, Heindl NR, Ott JA, den Blaauwen T and Bulgheresi S. Size-independent symmetric division in extraordinarily long cells. *Nature Communications 5:4803. doi: 10.1038/ncomms5803*

Scientific or technical background required for work program

We are looking for a student at the Master 2 level with a university cursus in prokaryotic cell biology, microbiology, and/or molecular biology. For the technical background, previous experience in molecular biology methods, immune-histo chemistry and light microscopy would be of big advantage. Previous work with methanogens, archaea, or anaerobic organisms is not necessary.

Title of the work program 27

Intracellular traficking of IgA protease of Pathogenic Isolates of *Neisseria meningitidis*

Description of the work program

Neisseria meningitidis is an exclusive human bacterium. Acquisition of this bacterium often leads to asymptomatic colonization (carriage) and rarely results in invasive disease (mainly septicaemia and meningitis) associated with extensive cell injury and tissue damage. Strains associated with invasive infections belong to a small number of genetic lineages referred to as hyper-invasive clonal complexes. Among these groupings, isolates that belong to the clonal complex ST-11 (cc11) positively correlated with fatal outcome of the disease and higher virulence in mice. We have previously shown that epithelial cells infected with cc11 isolates, but not asymptomatic isolates, exhibited features of apoptosis in later steps of infection. Apoptotic cell death promoted by cc11 isolates resulted from the nuclear cleavage of NF-κB mediated by a secreted form of the meningococcal IgA protease. Recent work in the lab showed intracellular trafficking of this bacterial protein through retrograde pathway to the endoplasmic reticulum and the interaction of IgA protease with the nuclear transport machinery of the host cell. Nevertheless, the mechanism responsible of the exit of IgA protease from the endoplasmic reticulum to the cytoplasm remains unknown.. This project aims to investigate the intracellular trafficking of IgA protease within epithelial cells to determine the way this protein crosses the endoplasmic reticulum to reach the cytoplasmic compartment.. This project will be performed within the Invasive Bacterial Infections Unit (IBI) that carry the National Refrence Center for Meningococci. This project relies on the use of meningococcal clinical isolates and mutants constructed in the unit as well as human epithelial cell lines established for meningococcal infection and several protease of cc11. Molecular and cell biology approaches will be involved.

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

1. Deghmane A-E, Veckerlé C, Giorgini D, Hong E, Ruckly C, Taha M-K. Differential modulation of TNF-alpha-induced apoptosis by Neisseria meningitidis. PLoS pathogens. 2009;5:e1000405.

2. Deghmane AE, El Kafsi H, Giorgini D, Abaza A, Taha MK. Late repression of NF-kappaB activity by invasive but not non-invasive meningococcal isolates is required to display apoptosis of epithelial cells. PLoS Pathog. 2011;7(12):e1002403. PMCID: 3228807.

3. Besbes A, Le Goff S, Antunes A, Terrade A, Hong E, Giorgini D, et al. Hyperinvasive Meningococci Induce Intra-nuclear Cleavage of the NF-kappaB Protein p65/RelA by Meningococcal IgA Protease. PLoS Pathog. 2015;11(8):e1005078.

Scientific or technical background required for work program

Title of the work program 28

Actin-activated bacterial nucleotidyl cyclase toxins

Description of the work program

Bacterial adenylate cyclase toxins are potent virulence factors that synthesize cAMP to modulate or disable the function of the host cell. *Pseudomonas aeruginosa*, an opportunistic human pathogen that causes severe acute infections in immunocompromised individuals and is a major cause of chronic infections in cystic fibrosis patients, encodes an adenylate cyclase toxin, called ExoY [1]. Together with 3 other proteins (ExoS, ExoT, and ExoU), ExoY is injected directly into the host cell utilizing the type III secretion system (T3SS) where they contribute to virulence of the pathogen [for review see [2, 3]].

Recent results show that substrate specificity of ExoY is not restricted to ATP as ExoY was shown to promote the intracellular accumulation of cAMP and cGMP, cCMP as well as cUMP.

In order to prevent detrimental effects resulting from the catalytic activity of ExoY inside the bacterial host, the protein is kept inactive inside the bacterial cell and acquires catalytic activity only after its delivery to the eukaryotic host cell through its interaction with a eukaryotic cofactor. We recently identified actin as said cofactor in our laboratory [4]. Actin is ubiquitously and abundantly present in eukaryotic cells and as such an appropriate indicator for the arrival of the bacterial toxin in the infected host. We also showed that the ExoY-like adenylate cyclase from *Vibrio nigripulchritudo*, despite its only distant relatedness to *P. aeruginosa* ExoY, is also activated by actin. These results suggest the presence of a group of actin-activated nucleotidyl cyclases (AA-NC). The two AA-NCs that we characterized, show important differences concerning their activation mechanism and substrate specificity.

To obtain further inside into the group of AA-NCs, the proposed project envisages the characterization of an ExoY-like protein from the genus *Proteus*. This genus harbors species (*P. vulgaris, P. mirabilis, P. penneri*), which are opportunistic human pathogens often responsible for urinary tract infections.

During work on this project, methods of molecular biology, biochemistry and bacteriology will be employed in our laboratory.

References:

1. Yahr, T. L., Vallis, A. J., Hancock, M. K., Barbieri, J. T. & Frank, D. W. (1998) ExoY, an adenylate cyclase secreted by the Pseudomonas aeruginosa type III system, *Proc Natl Acad Sci U S A.* **95**, 13899-904.
2. Engel, J. & Balachandran, P. (2009) Role of Pseudomonas aeruginosa type III effectors in disease, *Curr Opin Microbiol.* **12**, 61-6.
3. Hauser, A. R. (2009) The type III secretion system of Pseudomonas aeruginosa: infection by injection, *Nat Rev Microbiol.* **7**, 654-65.
4. Belyy, A., Raoux-Barbot, D., Saveanu, C., Namane, A., Ogryzko, V., Worpenberg, L., David, V., Henriot, V., Fellous, S., Merrifield, C., Assayag, E., Ladant, D., Renault, L. & Mechold, U. (2016) Actin activates Pseudomonas aeruginosa ExoY nucleotidyl cyclase toxin and ExoY-like effector domains from MARTX toxins, *Nat Commun.* **7**, 13582.

Keywords:

bacterial toxins, nucleotidyl cyclase, cNMP, actin, ExoY

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

- Belyy, A., Raoux-Barbot, D., Saveanu, C., Namane, A., Ogryzko, V., Worpenberg, L., David, V., Henriot, V., Fellous, S., Merrifield, C., Assayag, E., Ladant, D., Renault, L. & Mechold, U. (2016) Actin activates Pseudomonas aeruginosa ExoY nucleotidyl cyclase toxin and ExoY-like effector domains from MARTX toxins, *Nat Commun.* **7**, 13582

- Raoux-Barbot, D., Belyy, A., Worpenberg, L., Montluc, S., Deville, C., Henriot, V., Velours, C., Ladant, D., Renault, L. and Mechold, U. (2018) Differential regulation of actin-activated nucleotidyl cyclase virulence factors by filamentous and globular actin. *PLoS One*, **13**, e0206133.

Scientific or technical background required for work program

The candidate should have a solid knowledge in molecular biology and biochemistry, good English language skills, should be rigorous and organized with the ability to take initiative and should be eager to learn new methods.

Title of the work program 29

Validation and application of a novel SNP-based genotyping tool for *P. vivax* in samples from longitudinal cohorts and cross-sectional studies

Description of the work program

The Malaria: Parasites and Hosts Unit at Institut Pasteur in Paris, directed by Prof Ivo Mueller, conducts interdisciplinary field-based, laboratory-based and computational research with the aim of controlling and eliminating the transmission of *P. vivax* malaria.

Plasmodium vivax causes persistent and recurrent infections, mostly due to relapses of hypnozoites from the liver. To better understand the biology of *P. vivax* it is necessary to determine whether these infections are caused by a relapse or a new infection. The Unit has developed a novel amplicon deep sequencing (AmpSeq) tool targeting highly polymorphic genomic regions to address this question. In the proposed project, the student will assess the performance of AmpSeq in field samples from longitudinal cohorts and cross-sectional studies.

We are searching for enthusiastic students from a broad range of backgrounds. This project will provide experience working in a molecular epidemiology lab, from benchtop to desktop. The student will learn all the important considerations to design and run our amplicon sequencing workflow. That includes:

- DNA sample QC
- Libraries preparation and QC
- Sequencing by iSeq100 and/or MiSeq
Sequencing analysis: QC, demultiplexing, multi-locus haplotype inference

Tutor/supervisor

First name, Last name	Michael White
Phone	
E-mail	michael.white@pasteur.fr
Profile on http://www.researchgate.net/ (if applicable):	https://research.pasteur.fr/en/member/fr-michael-white/

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

https://research.pasteur.fr/en/team/malaria-parasites-and-hosts/

Scientific or technical background required for work program

Skills required
- Conventional PCR and qPCR
- DNA Electrophoresis
- DNA quantitation by any of these methods: Nanodrop or Qubit
- Basic knowledge of sequencing (Sanger and Next-Generation Sequencing)*
- Basic understanding of sequences analysis using BLAST, CLUSTAL*
- Basic commands in R*

* Desirable but not mandatory.

Title of the work program 30

Using mathematical models to design serological surveillance tools for the elimination of *Plasmodium vivax* malaria

Description of the work program

The Malaria: Parasites and Hosts Unit at Institut Pasteur in Paris, directed by Prof Ivo Mueller, conducts interdisciplinary field-based, laboratory-based and computational research with the aim of controlling and eliminating the transmission of *P. vivax* malaria.

The Unit and our collaborators have developed diagnostic technologies for detecting *P. vivax* malaria parasites by quantitative measurement of antibody responses. In the proposed project, the student will utilise a mathematical model of *P. vivax* transmission (also developed within the Unit) to assess the potential contribution that serological surveillance may make towards malaria elimination.

We are searching for enthusiastic students from a broad range of backgrounds. This project will provide experience working on mathematical and statistical models in close cooperation with field and laboratory based scientists. The student will gain experience in the following areas:

- Statistical analysis of serological data.
- Statistical analysis of epidemiological data.
- Mathematical modelling of infectious disease.
- Implementation of computational models in C++.

Tutor/supervisor

First name, Last name	Michael White
Phone	
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Profile on http://www.researchgate.net/ (if applicable):	https://research.pasteur.fr/en/member/fr-michael-white/

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

https://research.pasteur.fr/en/team/malaria-parasites-and-hosts/

Scientific or technical background required for work program

Skills required

- Experience of working with mathematical and/or statistical models.
- A strong interest in infectious disease epidemiology.
- Knowledge of a statistical programming language (preferably R).
- Ability to collate and analyse data, interpret and present results to a high standard using a range of specialised research techniques.

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Title of the work program 1 international

P. falciparum **lumefantrine resistance in French Guiana: status and evolution.**

Description of the work program

The laboratory of Dr. Lise Musset focuses on resistance of malaria parasites in the Guiana Shield/South America. As National Reference Center for Malaria for 30 years and WHO Collaborating Center for 8 years, the laboratory has a long-term interest in optimizing the use of antimalarial drugs. *Plasmodium spp.* parasites circulating in South America present many original aspects in terms of resistance and molecular markers. In this part of the world, resistant parasites appear *de novo* and *in situ*. Also, evolution of resistance and associated genotypes usually differ from those observed in Africa and South-East Asia. Our laboratory conducts research programs on *P. falciparum* in this area to explore: i) resistance mechanisms to antimalarial drugs, ii) evolution under drug pressure and iii) *in vivo* and *in vitro* phenotypes against currently used or future antimalarial drugs. Please visit our website http://www.pasteur-cayenne.fr for more information about projects and recent publications. Successful candidate will explores resistance to lumefantrine, an antimalarial drug combined with artemether actually recommended to treat *P. falciparum* infection in the Amazonian region. This issue will be investigated using French Guianan strains; high throughput genotyping methods and will be associate to a large part of *in vitro* multiplication of parasites and phenotyping methods.

Tutor/supervisor

First name, Last name	**Dr Lise Musset**
Phone	**+594 594 29 68 40**
E-mail	**lmusset@pasteur-cayenne.fr**
Profile on http://www.researchgate.net/ (if applicable):	**Lise Musset**

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

· **Musset L***, Heugas C*, Naldjinan R, Blanchet D, Houze P, Abboud P, Volney B, Walter G, Lazrek Y, Epelboin L, Pelleau S, Ringwald P, Legrand E, Demar M, Djossou F. Highlighting of *P. vivax* resistance to chloroquine in French Guiana during a therapeutic efficacy evaluation. *Antimic Agents Chemother*, **2019**. *In press.*

· Douine M, Lazrek Y, Blanchet D, Pelleau S, Chanlin R, Corlin F, Hureau L, Volney B, Hiwat H, Vreden S, Djossou F, Demar M, Nacher M, **Musset L**. Predictors of antimalarial self-medication in illegal gold miners in French Guiana: a pathway towards artemisinin resistance. *J Antimicrob Chemother*, **2018**. 73: 231-239.

· Faway E, **Musset L**, Pelleau S, Volney B, Casteras J, Caro V, Menard D, Briolant S, Legrand E. *Plasmodium vivax* multidrug resistance-1 gene polymorphism in French Guiana. *Malar J*. **2016**. 15: 540.

· Pelleau S, Moss EL, Dhingra SK, Volney B, Casteras J, Gabryszewski SJ, Volkmand SK, Wirth DF, Legrand E, Fidock DA*, Neafsey DE* & **Musset L**. Adaptive evolution of malaria parasites

in French Guiana: reversal of chloroquine resistance by acquisition of a novel *pfcrt* mutation. *Proc Natl Acad Sci USA*, **2015**. 112: 11672-11677.

Scientific or technical background required for work program

We are seeking a highly motivated individual. Skills in *in vitro* cultivation of malaria parasites would be appreciated.
- Adequate understanding of study design
- Statistics and data analysis.
- Written and communication skills.
- Ability to be self-guided
- Work effectively
- Flexibility and adaptability
- A sense of humor

This project is open to students enrolled in a M2 or PhD program.