Erasmus+

INSTITUT PASTEUR
EDUCATION

Projects 2024-2025

RESEARCH CENTRE

Legal name: **Institut Pasteur**

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

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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 600 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 77 different countries come to perfect their skills or conduct their Master or Doctoral trainings in the Institute's laboratories.

Description of the work program(s)

See projects on following pages

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

The laboratories at Pasteur have proposed 24 projects for Erasmus internships.
Students may also contact other laboratories at Pasteur to apply for an internship, even if the laboratories have not presented a project (https://research.pasteur.fr/en/).

FACILITIES (not compulsory for the host centre)

- **Accommodation**: a limited number of rooms for rent are reserved for Pasteur at the student residence "Cité Universitaire " http://www.ciup.fr/
- **Canteen**: partially subsidized canteen is available on the Pasteur Campus
- **Additional salary:** additional salary of approximately 600 euros/month (depending on the number of working days) is paid by the host lab (4.05 euros/hour, 7 hours/ day)

Title of work program 1

Validation of genetic modifiers for neurogenetic disease using simple model organism Caenorhabditis elegans

Description of the work program

Mitochondria are double membrane bound organelles that act as metabolic hubs and signaling platforms, involved in essential cellular processes. Mutations in mitochondrial genes cause a pleiotropic spectrum of clinical disorders with genetic, morphological and biochemical defects (1-2). Unfortunately, there are currently no cures for mitochondrial diseases and while next generation sequencing allowed significant progress towards the characterization of their genetic landscape, the explanation of the highly variable phenotypes observed even among individuals carrying the same pathogenic mutation is still lacking. For this reason, we are interested in understanding the mechanisms that regulate mitochondrial dynamics to better find ways to rebalance this pathway in genetic diseases. We decided to use a recently developed imaging and informatics pipeline available in the lab (3) to discover new genes that can restore mitochondrial form and function in cells modeling neurological disease.

In this project, the student will participate in the validation of the hits that affect mitochondrial functions by using *C. elegans*. Indeed, the student will validate the candidates' effect on this simple biological models' gene by using dsRNA feeding libraries to generate robust loss-of-function phenotypes. the **molecular and cellular basis** of the strains will be monitored using state-of-the-art technologies already running in the lab (blue-native PAGE, measurements of oxygen consumption, mitochondrial membrane morphology and potential, cell growth and confocal/super-resolution microscopy). Moreover, many robust **behavioral assays** have been developed for *C. elegans*, making analysis of behavioral mutants feasible and offering the promise of understanding the mechanisms underlying a whole animal's behavior at the molecular and cellular levels.

These new models will allow us to validate/reject our candidates that we identified and to take a first step towards the deciphering of the specific role played by the genetic variants underpinning mitochondrial disease pathogenesis and open new therapeutic avenues.

Tutor/supervisor

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

(1) Pujol, C., Legrand, A., Parodi, L., Thomas, P., Mochel, F., Saracino, D., Coarelli, G., Croon, M., Popovic, M., Valet, M., et al. (2021). Implication of folate deficiency in CYP2U1 loss of function. Journal of Experimental Medicine 218, e20210846. 10.1084/jem.20210846.

(2) Pujol, C., Lebigot, E., Gaignard, P., Galai, S., Kraoua, I., Bault, J.-P., Dard, R., Youssef-Turki, I.B., Omar, S., Boutron, A., et al. (2022). MPC2 variants disrupt mitochondrial pyruvate metabolism and cause an early-onset mitochondriopathy (In Review) 10.21203/rs.3.rs-2069607/v1.

(3) Cretin, E., Lopes, P., Vimont, E., Tatsuta, T., Langer, T., Gazi, A., Sachse, M., Yu-Wai-Man, P., Reynier, P., and Wai, T. (2021). High-throughput screening identifies suppressors of mitochondrial fragmentation in OPA1 fibroblasts. EMBO Mol Med 13. 10.15252/emmm.202013579.

Scientific or technical background required for work program

Basic knowledge of cell and molecular biology, rigour, curiosity.

Title of work program 2

Human iPSC-derived microglia and their roles at early stages of Alzheimer's Disease

Description of the work program

Microglia play critical roles in brain development and brain homeostasis, as well as the development of degenerative disorders including Alzheimer's disease (AD). In AD, accumulation of the β-amyloid (Aβ) peptide leads to the formation of amyloid plaques, occurs within the brain and represents one of the main hallmarks of the disease. However, the direct correlation between the levels of amyloid plaques and the progression of AD is unknown. It is important to identify the molecular mechanisms that are potentially capable of protecting against the onset of AD. The loss of cholinergic tone is hypothesized to be responsible for cognitive decline observed in AD patients. Current medication is restricted to enhancing cholinergic signaling for symptomatic treatment of these patients. There is an urgent need for more sophisticated models of AD that closely represent the main features and changes that occur in the brain of patients. The model with human-derived induced pluripotent stem cells (iPSC) is a powerful tool for elucidating the mechanisms which underly the pathogenesis of AD and provide the means for the development of curative measures in the future. In our laboratory, we transplant human brain cells derived from iPSC into the mouse brain to investigate the mechanisms by which reprogrammed human cells integrate into the brain of mouse neonates (P0-P1) (PMID: 30643170) and embryos (E17.5) (PMID: 37048140). Aβ production is achieved with viral transduction of human amyloid precursor protein harboring the well-known pathogenic mutations (hAPP-SLA, Swedish, London, and Austrian) to trigger Aβ deposition in targeted brain regions (PMIDs: 27999185; 27522251). We currently analyze the reactivity of human microglia to Aβ to better understand how these cells can regulate AD pathogenesis at early stages of the disease. Our experimental in-vitro and in-vivo approaches include immunofluorescence, pharmacology, cell mapping and single-cell transcriptomics techniques. Nicotinic cholinergic acetylcholine receptors (nAChR) regulate critical periods of brain maturation during the prenatal and early postnatal periods and are widely expressed by neurons and microglia. These receptors are also involved in AD (PMID: 25514383). *CHRFAM7A* is a human-specific nAChR gene which represents a partial duplicate of CHRFAM7A that can modulate the function of α7-nAChR. Since Aβ binds directly to the alpha7 subunit in neurons (PMID: 34067314), the α7-nAChR and its human-specific modulator are important cellular targets. We are currently investigating the interactions between Aβ and nAChR as well as their role in pathogenesis of AD by using mouse brain grafted by both, the human neurons and microglia. The cellular functions that are altered in AD can be studied by using iPSC-derived cells in vitro which can in turn provide initial insights into the in-vivo mechanisms. Our developed in-vitro and in-vivo models and the availability of diverse genetic tools provide the means, in Master 2 Project, to study the interactions between Aβ and nAChR as initial steps to determine if the genetic alterations of *CHRFAM7A* result in a loss of receptor function. This project will further allow to determine if the Copy Number Variations in this gene that are found in patients are linked to the severity of AD.

Tutor/supervisor

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Selected publications or patents of the Research Group offering the work program (2020-2024)

Llach Pou M, Thiberge C, Pons S, Maskos U and Cloëz-Tayarani I (**2024**) *CHRFAM7A* overexpression in human iPSC-derived Interneurons dysregulates α7- nAChR surface expression and alters response to oligomeric β-amyloid peptide. *bioRxiv* 2024.06.04.597325.

Llach Pou M, Thiberge M, Van der Zwan M, Pons S, Maskos U and Cloëz-Tayarani I (**2023**). Developmental Changes of Human Neural Progenitor Cells Grafted into the Ventricular System and Prefrontal Cortex of Mouse Brain in Utero. *Cells* 12(7), 1067.

Thiberge C, LLach Pou M, Vitrac A, Maskos U and Cloëz-Tayarani I (**2022**). Humanized chimeric mouse models to study human neural development. *NeuroMethods* 185.Chapter 8, 135-158.

Vitrac A, Pons S, Balkota M, Lemière N, Rais C, Bourgeois JP, Maskos U, Bourgeron T and Cloëz-Tayarani I (**2020**). A chimeric mouse model to study human iPSC-derived neurons: the case of a truncating SHANK3 mutation. *Sci Rep* 10, 13315.

Scientific or technical background required for work program

Scientific background: Molecular and cellular neuroscience, stem cell research

Technical skills: Cell culture, brain slicing for immunofluorescence staining, confocal microscopy

Other Skills**:** Communication, teamwork and adaptability

Title of work program 3

Deep learning for time-calibration of pathogen phylogenies

Description of the work program

Phylodynamics[1] bridges the gap between traditional epidemiology and pathogen genomes, by inferring epidemiological parameters (such as the expected number of secondary infections Re) from *pathogen transmission trees*. The internal nodes of these trees represent pathogen transmissions from a donor to a recipient individual, while the leaves correspond to pathogen sampling events. These transmission trees are often approximated by *time-scaled phylogenetic trees*[2], inferred from pathogen genomic sequences, which are sampled from infected individuals. In a pathogen phylogenetic tree the branches represent pathogen evolution and are measured in numbers of accumulated mutations (divided by the sequence size). Time-scaling combines the information coming from the tree branch lengths and the tip sampling dates to transform the phylogenetic tree so that its branches become measured in time. A time-scaled tree can answer such questions as when the epidemic started (the date of the root of the tree) and when certain transmissions happened (the dates of internal nodes).

Time-scaling is possible as many pathogens, especially viruses, quickly accumulate mutations between their transmissions, and because their mutation rate is roughly proportional to time (the *strict molecular clock* [2],). While many model-based tools are available for time-scaling trees under the strict and more complex clock models, this internship project's goal will be to investigate whether appropriate clock model detection and time-scaling can be also achieved with deep learning, and how it can help in epidemic surveillance tasks. *Deep learning* is currently revolutionizing different aspects of pathogen phylodynamics (e.g., epidemiological parameter estimation[3]), providing almost instantaneous inference for large data sets and computationally intractable models.

Specific tasks:

- perform a literature overview on different molecular clock models
- generate simulated data sets for different molecular clock and pathogen evolution settings
- develop a deep-learning architecture for this data and the performance metrics and train the deep learning time-scaler
- apply the deep-learning time-scaler to real pathogen data sets

As part of this project, the student will develop skills in designing and implementing deep learning tools for analyzing pathogen sequence data, designing bioinformatics workflows and performing calculations on a computational cluster, as well as in scientific writing and presentation.

In the long run, this project can be continued as a PhD project focussing on deep learning for phylodynamics.

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1 Volz EM, Koelle K, Bedford T. Viral phylodynamics. PLoS Comput Biol. 2013;9(3):e1002947. doi:10.1371/journal.pcbi.1002947

2 Ho SYW, Duchêne S. Molecular-clock methods for estimating evolutionary rates and timescales. Mol ecol. 2014. doi:10.1111/mec.12953

3 Voznica J, Zhukova A, Boskova V et al. Deep learning from phylogenies to uncover the epidemiological dynamics of outbreaks. Nat Commun. 2022;13:3896. doi:10.1038/s41467-022-31511-0

Tutor/supervisor

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

1. Featherstone, L. A. et al. (2024) Clockor2: Inferring global and local strict molecular clocks using root-to-tip regression. Systematic biology
2. Zhukova, A. et al. (2023). Fast and Accurate Maximum-Likelihood Estimation of Multi-Type Birth–Death Epidemiological Models from Phylogenetic Trees. Systematic Biology
3. Holtz et al. (2023). Integrating full and partial genome sequences to decipher the global spread of canine rabies virus. Nature Communications
4. Voznica et al. (2022). Deep learning from phylogenies to uncover the epidemiological dynamics of outbreaks. Nature Communications
5. Duchene S et al. (2020) Temporal signal and the phylodynamic threshold of SARS-CoV-2. Virus evolution
6. Ishikawa, S. A., Zhukova, A. et al. (2019). A fast likelihood method to reconstruct and visualize ancestral scenarios. Molecular biology and evolution

Scientific or technical background required for work program

We are looking for a candidate who is fluent in English, has a training in deep learning, knows how to program in python (or another programming language) and is interested in applying their computational skills to epidemiology.

Title of work program 4

Structural characterization of toxin complexes from Gram-positive bacteria

Description of the work program

This integrative project aims to investigate a novel class of bacterial effectors called LXG-toxins by combining different techniques such as Cryo-Electron Microscopy (Cryo-EM), protein crystallization, biophysical analyses as well as functional assays *(in vitro* and *in vivo*).

Increasing evidence indicate that LXG toxins act on shaping communities of Gram-positive bacteria and can mediate host-invasion in pathogens. Despite the importance of LXG-complexes many key questions are still open including their architecture, secretion mechanism and insertion in host cells.

Secretion of the LXG-toxins from bacteria is performed by the specialized machine 'Type VII Secretion System' (T7SS). We previously determined the crystal structural of a T7SS subunit from the model bacterium *Bacillus subtilis*. Also, we successfully identified a novel LXG toxin from clinical isolates of the opportunistic bacterium *Streptococcus gallolyticus* (here indicated as Sgg).

In this project we will investigate the multi-protein complex formed by the LXG toxins secreted by *B. subtilis* and by Sgg. Recently we obtained a preliminary 3D model by Cryo-EM as well as the crystal structure of a sub-complex of the Sgg protein complex.

We will integrate these data into functional and biophysical assays, while keeping increase model resolution. We will also create specific mutants to specifically address the questions on LXG-complex assembly and secretion.

The project is directed by Dr. F. Gubellini and will take place in the Structural Microbiology Unit (Dir. Prof. P. Alzari) in the Structural and Chemistry Department at the Institut Pasteur. This project is also carried on in collaboration with other groups inside and outside the Institut Pasteur.

The student will perform different tasks including protein purification and characterization (including protein stability, protein-protein interactions, and functional assays). Structural investigation (including data collection and analysis) will be carried out at the state-of-the-art facilities available the Institut Pasteur's campus for Crystallography and Cryo-Electron Microscopy.

Tutor/supervisor

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

High resolution cryo-EM and crystallographic snapshots of the actinobacterial two-in-one 2-oxoglutarate dehydrogenase.Yang L, Wagner T, Mechaly A, Boyko A, Bruch EM, Megrian D, **Gubellini F**, Alzari PM, Bellinzoni M.Nat Commun. 2023 Aug 10;14(1):4851. doi: 10.1038/s41467-023-40253-6.PMID: 37563123

Characterization of TelE, a T7SS LXG Effector Exhibiting a Conserved C-Terminal Glycine Zipper Motif Required for Toxicity.
Teh WK, Ding Y, **Gubellini F**, Filloux A, Poyart C, Givskov M, Dramsi S.Microbiol Spectr. 2023 Aug 17;11(4):e0148123. doi: 10.1128/spectrum.01481-23.

The Antibacterial Type VII Secretion System of Bacillus subtilis: Structure and Interactions of the Pseudokinase YukC/EssB.
Tassinari M, Doan T, Bellinzoni M, Chabalier M, Ben-Assaya M, Martinez M, Gaday Q, Alzari PM, Cascales E, Fronzes R, **Gubellini F.**mBio. 2022 Oct 26;13(5):e0013422. doi: 10.1128/mbio.00134-22.

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

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

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

Scientific or technical background required for work program

Good background in protein biochemistry and molecular biology required; knowledge in structural biology and microbiology is a plus.

Title of work program 5

Characterisation of muscle stem and committed cells in normal and pathological conditions

Description of the work program

There are two projects, and the most suitable candidate will be chosen based on their experience for one of them: 1) Muscle stem cell properties in distinct anatomical locations possess unique features that confer differential resistance to pathologies. These properties will be investigated using a variety of technologies including omics/microscopy/biochemistry/cell biology/live imaging with mice or in vitro generated embryo-like structures from mouse and human pluripotent cells; 2) bioinformatics analysis of high dimensional data obtained from imaging or omics studies of muscle stem/niche cells in diverse conditions including regeneration/ageing/pathologies.

Tutor/supervisor

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

Baghdadi MB, Castel D, Machado L, Fukada S, Birk DE, Relaix F, *Tajbakhsh S** and Mourikis P* (2018). Notch/CollagenV/CalcR reciprocal signalling retains muscle stem cells in their niche. **Nature** doi: 10.1038/s41586-018-0144-9. *co-corresponding.

Baghdadi MB, Firmino, J, K. Soni, B. Evano, Di Girolamo D, Mourikis, P Castel D and *Tajbakhsh S* (2018). Notch-induced microRNA-708 regulates cell migration and maintains quiescent muscle stem cells in their niche. **Cell Stem Cell**, 23:859-868. doi: 10.1016/j.stem.2018.09.017. Epub 2018 Nov 8.

Hernando-Herraez H#, Evano E#, Stubbs T#, Commere PH, Clark S, Andrews S, *Tajbakhsh S**, and Reik W* (2019). Ageing affects DNA methylation and transcriptional cell-to-cell variability in muscle stem cells. https://doi.org/10.1101/500900. #co-first authors.; *co-corresponding. **Nature Communications** 10(1):4361. doi: 10.1038/s41467-019-12293-4.

Evano B, Khalilian, S, Le Carrou G, Almouzni G, and *Tajbakhsh S* (2020). Dynamics of asymmetric and symmetric divisions of muscle stem cells in vivo and on artificial niches. **Cell Reports**, 30(10):3195-3206.e7. doi: 10.1016/j.celrep.2020.01.097.

Grimaldi A, G Comai, S. Mella and *S Tajbakhsh* (2022). Identification of bipotent progenitors that give rise to myogenic and connective tissues in mouse. https://www.biorxiv.org/content/10.1101/2021.05.26.445757v1. **Elife**. 2022 Feb 28;11:e70235. doi: 10.7554/eLife.70235.

Di Girolamo D. *, M. Benavente-Diaz*, Murolo, M., A. Grimaldi, P. Thomas Lopes, B. Evano, M. Kuriki, S. Gioftsidi, V. Laville, J.Y. Tinevez, G. Letort, S. Mella, S. Tajbakhsh# and G. Comai# (2024). Extraocular muscle stem cells exhibit distinct cellular properties associated with non-muscle molecular signatures. https://www.biorxiv.org/content/10.1101/2023.03.10.532049v2. Development, 151(4): dev202144. doi: 10.1242/dev.202144. Epub 2024 Feb 21. *co-first authors; # co-corresponding.

Kuriki M, A. Korb, G. Comai and *S. Tajbakhsh* (2024). Interplay between Pitx2 and Pax7 temporally governs specification extraocular muscle progenitors. https://www.biorxiv.org/content/10.1101/2023.08.24.554745v1. **PLoS Genet.** 14;20(6):e1010935. doi: 10.1371/journal.pgen.1010935.

Scientific or technical background required for work program

The candidate should be highly motivated for an internship of for 6-12 months, and have some experience with either molecular/cell biology/histology/confocal microscopy/handling of mice ("wet"), or programming experience for bioinformatics analyses and processing of "omics" and imaging pipeline data ("dry").

Title of work program 6

Computational and Statistical Analysis of Clinical Metagenomics Data

Description of the work program

Goal: The goal of the project is to understand how individual species in the vaginal microbiota affect and are affected by the overall microbial community in this environment.

The proposed work is part of the Innovative Strategies for Perinatal Infection Risk-Reduction (InSPIRe) project. InSPIRe is a collaboration of clinical and research team working to identify biomarkers of perinatal infections and antibiotic resistance. The host laboratory at Institut Pasteur has sequenced 2000 vaginal swab collected from pregnant women. The proposed work involves the analysis of shotgun sequencing data in order to investigate community composition as well as the role of individuals species in the vaginal microbiota.
The student will receive close mentorship from the PI and bioanalyist on the team. They will use existing **Python analysis pipelines**, and learn to make their own, in order to expand upon the results (1) showing a link between the vaginal microbiota and pregnancy outcomes. The student will learn and apply **analysis and statistical methods for microbiota analysis**. Additionally, the project involves the assembly of individual species genomics (i.e. *Escherichia coli* or *Lactobacillus crispatus*) from metagenomic data, followed by the annotation and comparison of these genomes. An comparative analysis of assembled genomes will be combined with clinical data to generate knowledge about species that influence both healthy microbial communities and those which present a higher risk for infection and other adverse pregnancy outcomes.
At the end of the internship, the student will have gained a better understanding of structured Python programming, as well as the analytic and statistical tools for clinical metagenomic analysis. These skills, which are generally applicable to other metagenomic projects, are also highly transferable to fields such as bioinformatics, clinical research, and genomics.

Tutor/supervisor

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

1. Baud, A. *et al.* Microbial diversity in the vaginal microbiota and its link to pregnancy outcomes. *Sci Rep* **13**, 9061 (2023).

2. Baud, A. & Kennedy, S. P. Targeted Metagenomic Databases Provide Improved Analysis of Microbiota Samples. *Microorganisms* **12**, 135 (2024).

3. Boutouchent, N., Vu, T. N. A., Landraud, L. & Kennedy, S. P. Exploring the Vaginal Microbiome During Pregnancy: Microbial Diversity, E. coli pathogenicity, and Links to Urinary Tract Colonization. Preprint at https://doi.org/10.21203/rs.3.rs-4795447/v1 (2024).

4. Ruppe, E. *et al.* Prediction of the intestinal resistome by a three-dimensional structure-based method. *Nature microbiology* **4**, 112-+ (2019).

Scientific or technical background required for work program

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

Title of work program 7

Development and validation of a primers set for allelic profiling of *eae* and *fliC* among Enteropathogenic *Escherichia coli* (EPEC)

Description of the work program

This work program is part of a larger research project entitled "Evaluation of the potential use of intimin and flagellar antigen types as molecular markers for the diversity of enteropathogenic *Escherichia coli* (EPEC) populations", which was recently funded by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). The main goal of the project is to propose new molecular markers for EPEC clustering when whole genome sequencing (WGS) is not available.

The work program is part of the third work package (WP) proposed in the research project and focuses on the identification and validation of primer sets for the amplification and downstream sequencing of *fliC* and *eae* alleles, which encode the flagellin and the outer membrane protein intimin of *E. coli*, respectively, and have been proposed as markers for the diversity of EPEC. Based on the allelic diversity of these genes, identified in other WPs of the research project, the selected student will be involved in building a multisequence alignment and a consensus sequence for each gene and use the built consensus sequence of those regions as a template for PCR primers that can be used for amplification and Sanger sequencing of the PCR products.

In addition, the student will help testing the sensibility and specificity of the designed primers both *in silico* and *in vitro*. In the "wet lab" part of the program, the student will help with genomic DNA extraction, preparation of PCR reactions and gel migration of PCR products. If necessary, the student will be encouraged to discuss with the principal investigator (PI) how to optimize PCR conditions in case unspecific products are observed. As a final step, the selected student will help purifying the obtained amplicons and preparing them for shipping to a Sanger sequencing platform. The student will also be involved in interpreting the obtained sequencing results.

Finally, the student will be invited to contribute to the preparation of a manuscript summarizing the main findings of the research project and will be encouraged to present their contribution to the project in different forms (lab meetings, poster or oral presentation during the yearly department retreat,...), even after the end of their stay in the Research Group, planned to last 6 months. Support with both wet and dry lab aspects of the work program, as well as the theorical knowledge required to better understand the genomic aspects of EPEC, will be provided by the PI and the Research Group team.

Tutor/supervisor

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

The host Research Group (Enteric Bacterial Pathogens – BPE) at Institut Pasteur includes the National Reference Centers (NRC) for *E. Coli*, *Shigella* and *Salmonella* (CNR-ESS) and for *Vibrio* and Cholera (CNR-VC) as well a WHO Collaborating Center for *Salmonella* diversity. Among the research interests and public health activities of the Reasearch Group are the development of new bacterial typing and diagnostic tools for our focus pathogens. Below is a list of selected published work related to the development of molecular typing tools and the use of genomics for pathogen surveillance.

- Hawkey et al., Genomic perspective on the bacillus causing paratyphoid B fever. [Preprint under review, doi: 10.21203/rs.3.rs-4502330/v1]
- Nodari et al., Population structure and dynamics of antimicrobial resistance among historical enteropathogenic *Escherichia coli*. [Presented as ePoster Flash Talk (ALP1003) at ECCMID 2023, manuscript in preparation].
- Yassine et al., ShigaPass: an *in silico* tool predicting *Shigella* serotypes from whole-genome sequencing assemblies. Microb Genom. 2023 Mar;9(3):mgen000961. doi: 10.1099/mgen.0.000961.
- Lefèvre et al., Rapid emergence of extensively drug-resistant *Shigella sonnei* in France. Nat Commun. 2023 Jan 28;14(1):462. doi: 10.1038/s41467-023-36222-8.
- Rouard et al., Contribution of microbial genomics to cholera epidemiology. C R Biol. 2022 May 11;345(1):37-56. doi: 10.5802/crbiol.77.
- Hawkey et al., Global population structure and genotyping framework for genomic surveillance of the major dysentery pathogen, *Shigella sonnei*. Nat Commun. 2021 May 11;12(1):2684. doi: 10.1038/s41467-021-22700-4.
- Zhou et al., The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. Genome Res. 2020 Jan;30(1):138-152. doi: 10.1101/gr.251678.119. Epub 2019 Dec 6.
- Wong et al., An extended genotyping framework for *Salmonella enterica* serovar Typhi, the cause of human typhoid. Nat Commun. 2016 Oct 5;7:12827. doi: 10.1038/ncomms12827.
- Fabre et al., CRISPR is an optimal target for the design of specific PCR assays for *Salmonella enterica* serotypes Typhi and Paratyphi A. PLoS Negl Trop Dis. 2014 Jan 30;8(1):e2671. doi: 10.1371/journal.pntd.0002671. eCollection 2014.

Scientific or technical background required for work program

- Good knowledge of molecular biology techniques, such as DNA extraction, preparation and interpretation of PCR reactions, amplicon purification, are essential, basic bioinformatics knowledge is a bonus.
- Basic knowledge on the particularities of working with bacterial DNA, including the preparation of bacterial cell cultures for DNA extraction, are important; knowledge of microbiology and bacterial genomics, even if limited, are a bonus.
- Good English communication skills are essential; basic French knowledge is a plus.
- Interest in public health and the surveillance of foodborne pathogens is a bonus.

Title of work program 8

Dynamics of DNA motion at a pluripotency gene using live imaging.

Description of the work program

In spite of intense investigations, it remains unclear whether and how the interaction between promoters and distant enhancers has a role in transcription. Until recently, this problem has been mainly addressed using techniques relying on cell fixation and DNA Next Generation Sequencing. Thanks to recent development of imaging technologies, it has become possible to observe the motion of DNA sequences and the production of mRNA molecules at a gene locus in live cells. In the 6-months of stay in the lab, the student will contribute to the production and analysis of a mouse embryonic stem cell line whose genome is engineered in such a way that the enhancer and promoter (or transcription, depending on time of start in the lab) of a specific pluripotency gene can be observed and monitored in space and time using **advanced fluorescence microscopy**. **Cell line engineering** will consist in the insertion of exogenous repetitive arrays at the endogenous gene locus using CRISPR-Cas9, followed by the random insertion using a PiggyBack system of the fluorescent reporter which is able to bind those sequences. The two steps will likely require optimization via different rounds of transfection and microscopic assessment. The aim is a **quantitative measurement of DNA motion and transcription**. This aim has specific requirements, whose leaning and understanding will be an important component of the traineeship.

The student will be directly supervised and work together with Sara Formichetti, a postdoc in the Unit for the Physics of Biological Function, directed by Thomas Gregor at Institut Pasteur in Paris (France). Sara will teach the student all the necessary techniques and guide her/him through all challenges encountered.

The candidate will be immersed in a highly interdisciplinary team, composed of biologists, physicists, engineers, and computer scientists. All have the same mission of a quantitative understanding of gene expression during development. The student will be encouraged to participate in discussions with all lab members. He/she will also be immersed in the lively and highly international environment of Institut Pasteur. According to project progress, there will be a possibility of an extension to up to 12-months in case of mutual agreement.

Tutor/supervisor

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

Chen, H. et al. Dynamic interplay between enhancer-promoter topology and gene activity. Nat. Genet. 50, 1296–1303 (2018).

Scientific or technical background required for work program

Scientific: molecular biology of transcription and chromatin in eukaryotes i.e. the transcription and mRNA cycle, the regulation of gene expression by non-coding regulatory sequences.

Technical:
- Experience with mammalian cell culture.
- Experience with standard molecular biology techniques: PCR, qPCR, cloning.

Motivation to work on a project that could involve a lot of technical troubleshooting.
Motivation to learn the biophysical approach to molecular biology and challenge her/himself with new advanced techniques, including single molecule microscopy.

The candidate will go through a VC interview to assess further his/her motivation and background.

Title of the work program 9

Investigating the influence of gut microbiota on the mesolimbic system and its response to nicotine

Description of the work program

While emerging evidence suggests a role for gut bacteria in the pathophysiology of substance use disorder (SUD), studies of their impact on brain and behavioral responses to drugs have been very limited so far. We previously showed that gut dysbiosis enhances the nicotine-induced activation of the mesolimbic system and alters nicotine's motivational properties in mice. However, the mechanisms linking gut bacteria to the mesolimbic system to influence its response to nicotine remain to be identified. Microbial products are suspected to play a role in this process by influencing gene expression, host immune and glial activation and synaptic signalling. In particular, short-chain fatty acids (SCFAs), the major microbial by-products of dietary fiber fermentation, are considered important targets for understanding the role of the gut microbiome in SUD as they notably regulate the secretion of gut hormones and can cross the blood-brain-barrier and affect epigenetic signaling in the brain. The objective of the project is to understand the role of SCFAs in the consequences of several types of gut dysbiosis on brain function in mice, in particular on nicotine-evoked neuronal activation in the mesolimbic system. We will further investigate the contribution of SCFAs-modulation of gut hormones in these effects. This research programme will contribute to identify the mechanisms by which gut microbiome alterations modulate the brain response to nicotine with the potential to improve our understanding of individual propensity to develop nicotine addiction.

Tutor/supervisor

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

Lakosa A*, Rahimian A*, Tomasi F*, Marti F, Reynolds LM, Tochon L, David V, Danckaert A, Canonne C, Tahraoui S, de Chaumont F, Forget B, Maskos U, Besson M$. Impact of the gut microbiome on nicotine's motivational effects and glial cells in the ventral tegmental area in male mice. Neuropsychopharmacology. 2023 May;48(6):963-974. doi: 10.1038/s41386-023-01563-x. PMID: 36932179.

Icick R, Forget B, Cloëz-Tayarani I, Pons S, Maskos U, Besson M$. Genetic susceptibility to nicotine addiction: Advances and shortcomings in our understanding of the CHRNA5/A3/B4 gene cluster contribution. Neuropharmacology. 2020 Oct 15;177:108234. doi: 10.1016/j.neuropharm.2020.108234. PMID: 32738310. https://hal-pasteur.archives-ouvertes.fr/pasteur-02936148

Forget B$, Icick R, Robert J, Correia C, Prevost MS, Gielen M, Corringer PJ, Bellivier F, Vorspan F, Besson M*$, Maskos U*$. Alterations in nicotinic receptor alpha5 subunit gene differentially impact early and later stages of cocaine addiction: a translational study in transgenic rats and patients. Prog Neurobiol. 2021 Feb;197:101898. doi: 10.1016/j.pneurobio.2020.101898. PMID: 32841724.

Besson M$, Forget B, Correia C, Blanco R, Maskos U$. Profound alteration in reward processing due to a human polymorphism in CHRNA5: a role in alcohol dependence and feeding behavior. Neuropsychopharmacology. 2019 Oct;44(11):1906-1916. doi: 10.1038/s41386-019-0462-0. PMID: 31288250.

Scientific or technical background required for work program

The candidate should have a strong background in Neuroscience, and preferentially a good knowledge of neuropsychiatric disorders and their neurobiological correlates, especially of the circuits and mechanisms of drug addiction. A background in immunology would be an important benefit. Ideally, the candidate would have previous training in rodent handling, behavioral testing and brain processing.

Title of work program 10

Actinobacterial cell division: understanding the molecular architecture of the divisome

Description of the work program

Understanding how one cell becomes two has always been a key question in cellular biology. Major differences exist between Archaea, Eukarya and Bacteria and even within the latter, the richness of cell shapes and cell wall compositions implies many specificities. Cell division has been a major target of antibiotics since the discovery of penicillin by Alexander Fleming in 1928. With the rise of antimicrobial resistance, which WHO deemed one of the ten utmost threats to global health in 2019, it is more crucial than ever to understand how cell division occurs in Bacteria.

Bacterial cell division requires the timely recruitment at the site of septation of a cell wall remodelling complex called the *divisome*, regulated both spatially and temporally to ensure the viability of the two daughter cells. characterising the molecular details of cell division has remained highly challenging due to the dynamic and membrane bound nature of these complexes. However, the recent technological developments in high-resolution cryo-microscopy, cutting-edge membrane technology and genetic tools has given a new impulse in the race towards unravelling the secrets of cell division in Bacteria at the molecular level.

In the lab, we use an integrative approach to study the detailed mechanisms of cell division: from *in vitro* biochemical characterisation and structure determination by crystallography and cryo-electron microscopy, to *in vivo* cell imaging and genetic engineering. We are especially interested in the medically relevant, human pathogen *Mycobacterium tuberculosis*, whose complex cell wall still remains mysterious. For our cellular studies we work with the non-pathogenic actinobacterial model organism *Corynebacterium glutamicum*. Our multidisciplinary perspective has recently been proven successful in gaining new insights in corynebacterial cell division [Martinez, et al., 2023; Sogues *et al.*, 2020].

The scientific outcomes will shed light on the regulation of a fundamental and cardinal process of bacterial cell biology. The benefits are diverse and range from an immediate knowledge of cell division to the opening of new concepts concerning the inner-workings of a living cell. Furthermore, since cell division is fundamental to all forms of life, a better understanding of how bacteria grow and divide at the molecular level is not only important for cell biology, but it is also expected to have a strong impact on biomedical research.

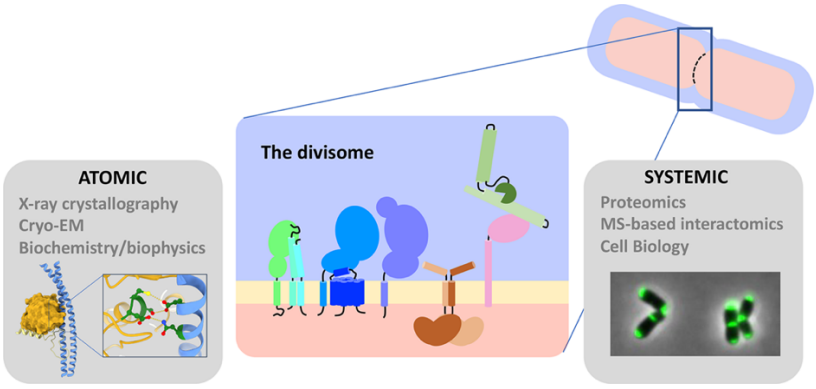

Figure 1: an integrative approach to understand corynebacterial cell division.

Tutor/supervisor

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

1. Martinez M, Petit J, Leyva A, Sogues A, Megrian D, Rodriguez A, Gaday Q, Ben Assaya M, Portela M, Haouz A, Ducret A, Grangeasse C, Alzari PM, Durán R#, Wehenkel A# (2023). Eukaryotic-like gephyrin and cognate membrane receptor coordinate corynebacterial cell division and polar elongation. 2023. Nature Microbiology 8, 1896–1910. # Corresponding author

2. Gaday Q, Megrian D, Carloni G, Martinez M, Sokolova B, Ben Assaya M, Legrand P, Brûlé S, Haouz A, Wehenkel A#, Alzari PM# (2022). Structural basis of FtsEX-independent RipA-mediated cell separation in Corynebacteriales. Proc Natl Assoc Sci USA, 119, e2214599119. #Corresponding author

3. Sogues, A., Martinez, M., Gaday, Q., Ben-Assaya, M., Graña, M., Voegele, A., VanNieuwenhze, M., England, P., Haouz, A., Chenal, A., Trepout, S., Duran, R., Wehenkel#, A. & Alzari#, PM. Essential dynamic interdependence of FtsZ and SepF for Z-ring and septum formation in Corynebacterium glutamicum. Nat Commun 11, 1–14 (2020). # Corresponding author.

Scientific or technical background required for work program

We are looking for a curious, motivated student, preferably studying for a University degree that provides her/him with general knowledge in either microbiology, molecular biology, biochemistry, biophysics or cell imaging. Previous lab experience would be beneficial. This internship represents an opportunity to be acquainted with a large range of techniques, and help us answer fundamentally and therapeutically important questions.

Title of the work program

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

Description of the work program

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

Tutor/supervisor

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

- Blachier, S. Intranasal delivery of a broadly neutralizing single domain antibody targeting ACE2 protects against SARS-CoV-2 infection. *BioRxiv* (2024). doi.org/10.1101/2024.10.11.617877
- Momboisse F, Nardi G, Colin P, Hery M, Cordeiro N, Blachier S, Schwartz O, Arenzana-Seisdedos F, Sauvonnet N, Olivo-Marin JC, Lagane B, Lagache T, **Brelot A**. Tracking receptor motions at the plasma membrane reveals distinct effects of ligands on CCR5 dynamics depending on its dimerization status. Elife. **2022** Jul 22;11:e76281. doi: 10.7554/eLife.76281.
- Gaelle Boncompain, Floriane Herit, Sarah Tessier, Aurianne Lescure, Elaine Del Nery, Pierre Gestraud, Isabelle Staropoli, Yuko Fukata, Masaki Fukata, Anne Brelot, Florence Niedergang, and Franck Perez. (2019). Targeting CCR5 trafficking to inhibit HIV-1 infection. Science Advances, Oct 16;5(10).
- Colin P, Zhou Z, Staropoli I, Garcia-Perez J, Gasser R, Armani-Tourret M, Benureau Y, Gonzalez N, Jin J, Connell BJ, Raymond S, Delobel P, Izopet J, Lortat-Jacob H, Alcami J, Arenzana-Seisdedos F, Brelot A, Lagane B. (2018). CCR5 structural plasticity shapes HIV-1 phenotypic properties. PLoS Pathog. 2018 Dec 6;14(12):e1007432.
- Brelot A, Chakrabarti LA (2018). CCR5 revisited: How mechanisms of HIV Entry govern AIDS pathogenesis J Mol Biol. 2018 Aug 17;430(17):2557-2589.
- Jin J, Momboisse F, Boncompain G, Koensgen F, Zhou Z, Cordeiro N, Arenzana-Seisdedos F, Perez F, Lagane B, Kellenberger E, Brelot A. (2018). CCR5 adopts three homodimeric conformations that control cell surface delivery. Science Signaling May 8;11(529).
- Jin J, Colin P, Staropoli I, Lima-Fernandes E, Ferret C, Demir A, Rogée S, Hartley O, Randriamampita C, Scott MG, Marullo S, Sauvonnet N, Arenzana-Seisdedos F, Lagane B, Brelot A. (2014). Targeting spare CC chemokine receptor 5 (CCR5) as a principle to inhibit HIV-1 entry. J Biol Chem. Jul 4;289(27):19042-52.

Scientific or technical background required for work program

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

Title of work program 12

Immune cell heterogeneity at the choroid plexus along post-natal development.

Description of the work program

During postnatal development, two linked systems of the body form in parallel: the brain, which grows incredibly quickly during this period, and the immune system, which needs to mature quickly as the body is, for the first time, exposed to the pathogens in the outside world. Can these maturation processes in the two systems be linked? To address this question, we study one of the brain borders, the choroid plexus, a tissue which produces the cerebrospinal fluid, a liquid carrying growth factors key for brain development, but also interacts with the peripheral immune system. In this project, we use scRNA-seq, imaging, and flow cytometry analyses in mouse models to study the heterogeneity of immune cells populating the CP from birth to adulthood.

Tutor/supervisor

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

1. L Travier, R Singh, D Sáenz Fernández, A Deczkowska, Microbial and immune factors regulate brain maintenance and aging, Current Opinion in Neurobiology. 2022.
2. Deczkowska A, David E, Ramadori P, Pfister D, Safran M, Li B, Giladi A, ... , Weiner A, Ben-Ari Z, Heikenwälder M, Elinav E & Amit I XCR1+ type 1 conventional dendritic cells drive liver pathology in non-alcoholic steatohepatitis, Nature Medicine. 2021.
3. Deczkowska A, Weiner A, Amit I. The Physiology, Pathology, and Potential Therapeutic Applications of the TREM2 Signaling Pathway. Cell. 2020.
4. Deczkowska A, Schwartz M. Targeting neuro-immune communication in neurodegeneration: Challenges and opportunities. J Exp Med. 2018.
5. Deczkowska A, (…), Amit I. Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. Cell. 2018.
6. Deczkowska A, Amit I, Schwartz M. Microglial immune checkpoint mechanisms. Nat Neurosci. 2018.
7. Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, (…) , Schwartz M. Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. Nat Commun. 2017.

Scientific or technical background required for work program

Must: be OK working with mice, speaking English, interest in neuroimmunology
Welcome: experience with flow cytometry, RNA sequencing, fluorescence and confocal imaging, experience with scRNA-seq analysis

Title of work program 13

Characterization of antibodies targeting poxvirus envelope proteins

Description of the work program

The Strutural Biology of Infectious Diseases is a junior unit dedicated to the study of poxviruses, a family of DNA viruses that includes important human pathogens such as variola or mpox viruses. Smallpox, which is the disease caused by the variola virus, has been the causative agent of epidemics for centuries until it was eradicated in the 70s after a long vaccination campaign. For safety reasons, the vaccination was interrumped 40 years ago, and the current population is no longer protected, raising concerns about the propagation of mpox or the reintroduction of smallpox as a biological weapon. For this reason, new vaccines need to be developed. They should be safe, easy to produce and confer long-lasting immunity. In our unit, we used the vaccinia virus as a model system to understand poxvirus biology. In particular, we are interested in understanding the fusion mechanism. In contrast to other viruses, poxviruses enter cells because of a complex of 11 proteins present on the viral envelope called the entry fusion complex (EFC). Although EFC components have been identified, the molecular mechanisms by which they act remain unclear. By combining biological and structural approaches, we aim to characterize the individual EFC components and how they interact and work. Our objective is to combine structural and immunological data to design new-generation vaccines.

Tutor/supervisor

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

Neutralization determinants on Poxviruses.
Vernuccio R et al. Viruses, 2023 https://doi.org/10.3390/v15122396

Production and Purification of Hantavirus Glycoproteins in Drosophila melanogaster S2 cells.
Meola A et al. Methods Mol Biol, 2024 DOI: 10.1007/978-1-0716-3666-4_1

MPXV Infection stimulates a more robust and durable neutralizing antibody response compared to MVA-BN vaccination.
Selverian CN et al. J Infec Dis., 2024. PMID: **39422181** DOI: 10.1093/infdis/jiae515

Mechanisms of tecovirimat antiviral activity and poxvirus resistance.
Vernuccio R et al. Res Sq. 2024 DOI: 10.21203/rs.3.rs-5002222/v1

Scientific or technical background required for work program

To achieve the prefixed objectives, knowledge in protein expression and purification methods is a necessary requirement. Some experience with cell handling will be a plus. BLI knowledge will be also appreciated. Skills in either X-ray crystallography or cryo-EM will be acquired on site according to the project evolution.

Title of work program 14

Host response to tuberculosis

Description of the work program

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is one of the deadliest diseases caused by a single infectious agent, alongside COVID-19. According to the latest WHO report, 10 million new TB cases were recorded, and the disease claimed 1.3 million lives in 2022. Despite considerable efforts, TB remains a major public health issue. There is still no fully effective vaccine against TB, and multidrug-resistant (MDR) strains of MTB continue to emerge. Combating tuberculosis, therefore, requires new strategies and a better understanding of host-pathogen interactions.

In our laboratory, we are developing several projects aimed at:

1. Finding new molecules that enhance the resistance or bactericidal functions of innate immune cells and/or improve the efficacy of anti-tuberculosis drugs,
2. Understanding and evaluating the long-term impacts of the disease on the body,
3. Studying the role of NK cells in TB.
4.

Depending on the length of the internship, candidates will work on one of these projects. They will use a combination of cellular biology, microbiology, and immunology techniques. They will notably learn how to isolate human cells from blood, differentiate these cells into macrophages (the main targets of the bacteria), and infect them with fluorescent MTB strains or attenuated bacteria. The interactions between MTB and its host will then be studied using advanced imaging techniques. The murine model of TB will also be used for some of these projects. Other approaches commonly used in the laboratory include genomics (RNA sequencing), and flow cytometry.

Tutor/supervisor

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

1 Maure, A. et al. A host-directed oxadiazole compound potentiates antituberculosis treatment via zinc poisoning in human macrophages and in a mouse model of infection. PLoS Biol. Apr 29;22(4):e3002259 (2024).

2 Giraud-Gatineau, A. et al. The antibiotic bedaquiline activates host macrophage innate immune resistance to bacterial infection. eLife 9, doi:10.7554/eLife.55692 (2020).

3 Bottai, D. et al. TbD1 deletion as a driver of the evolutionary success of modern epidemic Mycobacterium tuberculosis lineages. Nat Commun 11, 684, doi:10.1038/s41467-020-14508-5 (2020).

4 Coya, J. M. et al. Tri-mannose grafting of chitosan nanocarriers remodels the macrophage response to bacterial infection. J Nanobiotechnology 17, 15, doi:10.1186/s12951-018-0439-x (2019).

5 Groschel, M. I. et al. Recombinant BCG Expressing ESX-1 of Mycobacterium marinum Combines Low Virulence with Cytosolic Immune Signaling and Improved TB Protection. Cell Rep 18, 2752-2765, doi:10.1016/j.celrep.2017.02.057 (2017).

Scientific or technical background required for work program

- Strong motivation, scientific curiosity and interest in microbiology or immunology
- Good verbal and written English communication skills are expected
- Research experience in cell culture and/or animal manipulation is regarded very favorably

Title of work program 15

Functional characterization of *L. pneumophila* nucleomodulins

Description of the work program

Legionella pneumophila is an intracellular pathogen that replicates with eukaryotic cells, mainly aquatic protozoa. Genome analyses suggested that *L. pneumophila* acquired eukaryotic genes from its hosts during this co-evolution [1]. In the last years it has been shown that many of these so-called "eukaryotic-like" proteins are secreted virulence factors that are translocated via a Type-4 secretion system (T4SS) into the host cell[2]. Among the over 330 proteins described as T4SS substrates, 80% are encoded by genes thought to be acquired from eukaryotic hosts. Recent functional studies showed that several of these "eukaryotic like" proteins help *L. pneumophila* to subvert host functions to its advantage. Thus, molecular mimicry of eukaryotic proteins is an efficient strategy of *L. pneumophila* to intercept host responses and to promote its intracellular replication [3].

One of our main research questions is to understand how *L. pneumophila* subverts host functions to replicate in the host cell and to cause disease. We particularly focus on studying the effectors that can target the host cell nucleus and try to understand which nuclear functions this bacterium is able to manipulate to its advantage. We have already described two effectors that can cooperate at the chromatin level of the infected cell to change post-translational histone marks and thereby control the transcriptional response of the host cell [4]. An in-depth search for putative chromatin modifying effectors encoded by *L. pneumophila* led to the identification of additional putative nuclear effectors, called nucleomodulins [5].

In the here proposed project, the student will work on one of these putative nucleomodulins to learn whether it is secreted by the bacterium into the target cell, if it encodes chromatin modifying activity and if it modulates a nuclear pathway. He/she will work together with staff scientists and cell biology, microbiology and biochemical technologies will be used.

Tutor/supervisor

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

1. Cazalet, C. *et al.* Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat Genet* **36**, 1165–1173 (2004).
2. Mondino, S. *et al.* Legionnaires' Disease: State of the Art Knowledge of Pathogenesis Mechanisms of Legionella. *Annu Rev Pathol* **15**, 439–466 (2020).
3. Mondino, S., Schmidt, S. & Buchrieser, C. Molecular Mimicry: a Paradigm of Host-Microbe Coevolution Illustrated by *Legionella*. *Mbio* **11**, e01201-20 (2020).
4. Schator, D. *et al.* Legionella para-effectors target chromatin and promote bacterial replication. *Nat Commun* **14**, 2154 (2023).
5. Bierne, H. & Pourpre, R. Bacterial Factors Targeting the Nucleus: The Growing Family of Nucleomodulins. *Toxins (Basel)* **12**, 220–19 (2020).

Scientific or technical background required for work program

Technical skills:

Basic knowledge in:

- cell biology (cell line culture)
- microbiology (sterile techniques, plating methods)
- molecular biology (DNA extraction, plasmid preparation, cloning, PCR, agarose gel electrophoresis)
- biochemistry (western blots, SD-PAGE)

Non-technical skills:

- Good knowledge in English
- Teamwork skills

Title of work program 16

Deep characterization of the drinking water microbiome

Description of the work program

Despite the importance of the exposure to drinking water (DW) microbiomes for public health, current understanding of DW microbiomes and their link to water-quality parameters is still incomplete. DW microbiomes are understudied compared to other microbiomes. Thus, we develop a project that aims to answer the important questions: what are the relationships between bacteria, archaea, fungi and protists that constitute the DW microbiome, and what is the impact of key physiochemical water parameters? This knowledge is needed to understand the evolution of the DW quality while it is travelling through distribution systems to the end user. In particular, to adequately control opportunistic waterborne pathogens on building plumbing systems.

Opportunistic waterborne pathogens cause disease mainly in individuals having risk factors, such as advanced age, cancer or immunodeficiency, a proportion of the population that is steadily increasing. Many different bacteria can be present in DW, but most of the waterborne diseases and death can be attributed to three bacterial genera: *Legionella* spp, *Pseudomonas* spp and nontuberculous *Mycobacteria* (NTM). Our project aims to obtain an in-depth characterization of the distribution of these opportunistic waterborne pathogens in drinking water (DW) systems and to decipher the factors (biotic and abiotic) that are key to control them. To reach this aim, in collaboration with water of Paris, we collect samples from the water distribution system, and we analyze these samples using metagenomics and quantitative PCR: metabarconding for characterization of bacteria and eukaryots, for selected waterborne pathogens metabarcoding, digital PCR and shotgun (for selected samples). The main objective is to obtain a comprehensive description of this microbiome from source to tap focusing mostly on three bacterial genera: *Legionella* spp, *Pseudomonas* spp and non-tuberculous mycobacteria and in their associated amoeba hosts. We want to understand their frequence and distribution from source to tap and their relationships with amoeba.

Tutor/supervisor

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

Martyn JE, **Gomez-Valero L**, Buchrieser C, , The evolution and role of eukaryotic-like domains in environmental intracellular bacteria: the battle with a eukaryotic cell., FEMS Microbiol Rev 2022 Feb; ():2021

Pérez-Cobas AE, **Gomez-Valero L**, Buchrieser C, , Metagenomic approaches in microbial ecology: an update on whole-genome and marker gene sequencing analyses., Microb Genom 2020 Jul; (): .2020

Mondino S, Schmidt S, Rolando M, Escoll P, **Gomez-Valero L**, Buchrieser C, , Legionnaires' Disease: State of the Art Knowledge of Pathogenesis Mechanisms of Legionella., Annu Rev Pathol 2020 01; 15(): 439-466.2019

Gomez-Valero L, Chiner-Oms A, Comas I, Buchrieser C, Evolutionary dissection of the Dot/Icm system based on comparative genomics of 58 Legionella species, Genome Biology and Evolution, evz186.2019

Gomez-Valero L, Buchrieser C, Intracellular parasitism, the driving force of evolution of Legionella pneumophila and the genus Legionella., Genes Immun. 2019 May;20(5):394-402.2019

Gomez-Valero L, Rusniok C, Carson D, Mondino S, Pérez-Cobas AE, Rolando M, Pasricha S, Reuter S, Demirtas J, Crumbach J, Descorps-Declere S, Hartland EL, Jarraud S, Dougan G, Schroeder GN, Frankel G, Buchrieser C, , More than 18,000 effectors in the Legionella genus genome provide multiple, independent combinations for replication in human cells., Proc Natl Acad Sci U S A 2019 02; 116(6): 2265-2273.2019

Scientific or technical background required for work program

Desirable:

PCR
Dna Extraction
Programmation skills: R - Python

Title of work program 17

Epigenetic inheritance of stress responses and diet through the gut-germline axis

Description of the work program

While heritable traits are predominantly encoded in DNA, epigenetic mechanisms—including DNA methylation, histone modifications, and small RNAs—are increasingly recognized across organisms as carriers of heritable information (Cecere, 2021). The nematode *Caenorhabditis elegans* has been instrumental in demonstrating how germline-inherited small RNAs can alter progeny phenotype. For instance, we have elucidated how animals gradually lose their fertility across generations through the inheritance of small RNAs antisense to histone mRNAs (Barucci et al., 2020). However, while much research has focused on the transmission of small RNAs and histone modifications from the parental germline to the embryo and the phenotypic impact of this inheritance, little is known about soma-to-germline transfer of epigenetic information.

Unlike genetic inheritance, epigenetic inheritance is influenced by environmental factors such as diet, pathogens, and temperature. These external factors can potentially modify the parental germline's epigenetic state, impacting offspring phenotypes[6]. Yet, the mechanisms underlying environmentally induced epigenetic inheritance remain poorly understood. Specifically, it is unclear whether and how somatic cells exposed to stimuli (e.g., infection) can induce epigenetic changes in the germline and how these modifications evade the extensive epigenomic reprogramming that occurs in embryos.

Recent studies in nematodes, fruit flies, and mice have identified a gut-germline axis that transmits heritable information triggered by infections, diet, or gut microbiota. However, the molecular mechanisms underlying this gut-to-germline communication remain poorly understood. Although chromatin modifications have been implicated to some extent, it remains unclear whether and how histone modification changes induced by gut stimuli are communicated to the germline and passed on to future generations. Moreover, the implication of small RNAs in such soma-to-germline communication is scarce.

We hypothesize that evolutionarily conserved chromatin-based mechanisms and small RNAs can propagate the memory of somatic gene expression programs to the germline, enabling the transmission of stress resilience across generations.

Using a well-established *C. elegans* gut-bacteria system, we will investigate whether and how nutritional changes with different bacteria or bacterial infections will impact the germline chromatin, histone modifications, and small RNAs, and their impact on the next generations to promote stress resilience.

To study these epigenetic changes, we will adopt sequencing methods, such as Cut and Tag (to measure histone modifications) GRO-seq (to measure nascent transcription), and small RNA sequencing to identify loci that will memorize such environmental changes. Mutants of known chromatin factors will be used in combination with small RNA pathway mutants to study their requirement in the establishment and/or the maintenance of heritable changes.

This project is highly multidisciplinary and will allow the student to acquire diverse skills ranging from molecular biology (RT-qPCR and genomic methods), imaging (monitoring transcriptional responses to heritable small RNAs in living C. elegans animals by microscopy and FACS sorting).

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

Research articles:

Barucci G, Cornes E, Singh M, Li B, Ugolini M, Samolygo A, Didier C, Dingli F, Loew D, Quarato P, Cecere G. *Small-RNA-mediated transgenerational silencing of histone genes impairs fertility in piRNA mutants.* Nature Cell Biology. 2020 Feb;22(2):235-245.
Cover Article, free online text: https://rdcu.be/b1fm7

Quarato P, Singh M, Cornes E, Li B, Bourdon L, Mueller F, Didier C, Cecere G. *Germline inherited small RNAs facilitate the clearance of untranslated maternal mRNAs in C. elegans embryos*. Nature Communications. 2021 Mar 4;12(1):1441. doi: 10.1038/s41467-021-21691-6. PMID: 33664268

Cornes E, Bourdon L, Singh M, Mueller F, Quarato P, Wernersson E, Bienko M, Li B, Cecere G. *piRNAs initiate transcriptional silencing of spermatogenic genes during C. elegans germline development.* Developmental Cell. 2022 Jan 24;57(2):180-196.e7. doi: 10.1016/j.devcel.2021.11.025. Epub 2021 Dec 17. PMID: 34921763. Free article here: https://www.cell.com/developmental-cell/fulltext/S1534-5807(21)00989-8

Review articles:

Cecere G. *Small RNAs in epigenetic inheritance: from mechanisms to trait transmission*. FEBS Letters. 2021 Dec;595(24):2953-2977. doi: 10.1002/1873-3468.14210. Epub 2021 Oct 29.
PMID: 34671979. Free online article: https://febs.onlinelibrary.wiley.com/doi/10.1002/1873-3468.14210

Quarato P, Singh M, Bourdon L, Cecere G. *Inheritance and maintenance of small RNA-mediated epigenetic effects.* Bioessays. 2022 Jun;44(6):e2100284.
Free online article: https://doi.org/10.1002/bies.202100284

Scientific or technical background required for work program

The successful applicant should have good knowledge in molecular biological techniques (such as DNA/RNA preparation, PCR, RT-PCR, quantitative PCR, molecular cloning, western blotting). Experience in sequencing data analysis or bioinformatics and using the *C. elegans* model is not required but will be highly regarded. The applicant should be well-organized, highly motivated, enthusiastic about the project, willing to learn new techniques, and read scientific papers. Good English-language communication skills are required.

Title of work program 18

Using Machine-Learning for tracking individual neurons in the small cnidarian Hydra

Description of the work program

The small cnidarian Hydra possesses one of the simplest "brain" of the animal kingdom. Therefore, the tracking of all his individual neurons is possible and might lead to the first entire decoding of a brain (i.e. the understanding of how interacting neurons can integrate the environment's cues, compute the animal's state and trigger appropriate behaviors).
An important bottleneck for the long-term monitoring of neuronal activity in small animal systems like Hydra, is the robust tracking of neurons in the behaving animal (Figure). Single particle tracking in fluorescence microscopy is hindered by the difficult detection of fluorescent spots (neurons) over a noisy background, and their unpredictable motion related to animal behavior and deformation.
This project will aim at developing innovative algorithms based on machine-learning for the robust detection and tracking of neurons in behaving animals. The project will build on recent advances in deep-learning assisted detection of cells (Schmidt, U., Weigert, M., Broaddus, C., & Myers, G. (2018). Cell detection with star-convex polygons. In *Medical Image Computing and Computer Assisted Intervention–MICCAI 2018* & Reference 2) and the expertise of the Research Group in single-particle-tracking (Refs 1-6).

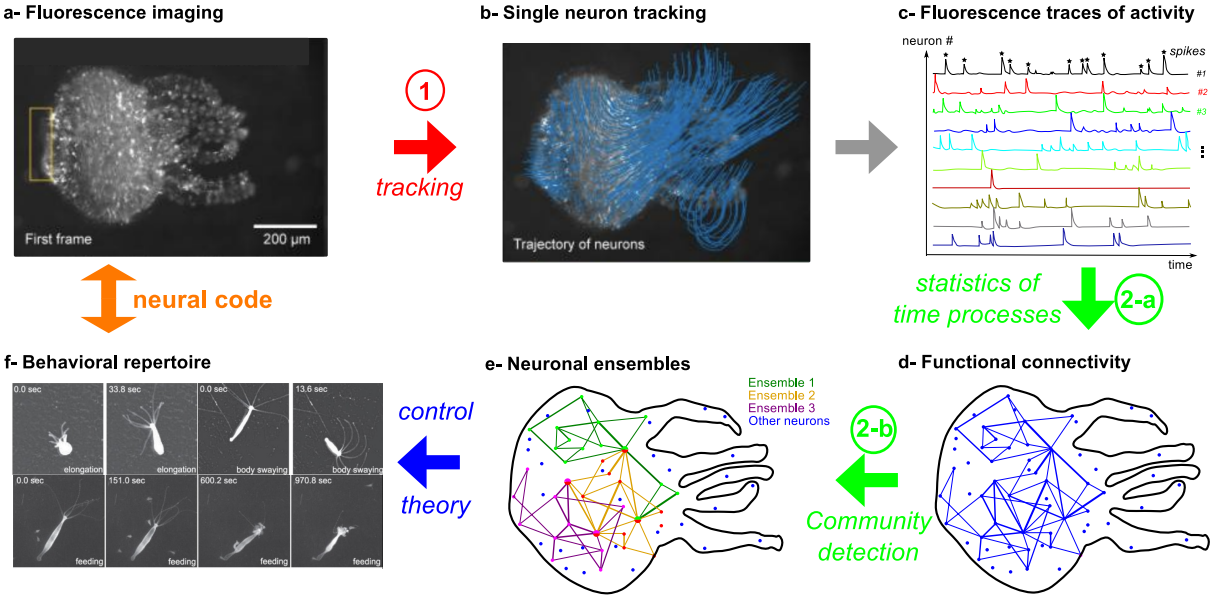

Figure 1: ***Breaking*** **the neural code of Hydra** consists in relating the sequential activity of neurons or ensemble of neurons, observed with fluorescence imaging (**a**) to each specific action of the animal (**f**), as they document significant relations of causality between the time series of individual neurons' activities. Our multi-step approach consists in (**b**) long-term, single particle tracking of $\approx 1000 - 2000$ neurons in freely-behaving and deforming animal (manual tracking over only 200 frames (20 s) is shown here (adapted from [3]), (**c**) extraction of individual fluorescence traces and spikes (highlighted with black stars for neuron #1), (**d**) statistical inference of neurons' functional connectivity (line thickness indicate connection weights) and, (**e**) clustering into significant neuronal ensembles. Finally, recasting the activity and functional connectivity of individual neurons in an optimal control theory framework will help to understand how the coordinated activity of hundreds to thousands neurons control the animal's state (**f**).

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

1- Reme, R., Newson, A., Angelini, E., Olivo-Marin, J. C., & Lagache, T. (2024, May). Particle Tracking in Biological Images with Optical-Flow Enhanced Kalman Filtering. In *2024 IEEE International Symposium on Biomedical Imaging (ISBI)* (pp. 1-5). IEEE.

2- Hanson, A., Reme, R., Telerman, N., Yamamoto, W., Olivo-Marin, J. C., Lagache, T., & Yuste, R. (2024). Automatic monitoring of neural activity with single-cell resolution in behaving Hydra. *Scientific Reports*, *14*(1), 5083.

3- Lagache, T., Hanson, A., Fairhall, A., & Yuste, R. (2020). Robust single neuron tracking of calcium imaging in behaving Hydra. *bioRxiv*.

4- Dupre, C., & Yuste, R. (2017). Non-overlapping neural networks in Hydra vulgaris. *Current Biology*, *27*(8), 1085-1097.

5- Chenouard, N., Bloch, I., & Olivo-Marin, J. C. (2013). Multiple hypothesis tracking for cluttered biological image sequences. *IEEE transactions on pattern analysis and machine intelligence*, *35*(11), 2736-3750.

6- De Chaumont, F., Dallongeville, S., Chenouard, N., Hervé, N., Pop, S., Provoost, T., ... & Olivo-Marin, J.-C. (2012). Icy: an open bioimage informatics platform for extended reproducible research. *Nature methods*, *9*(7), 690-696.

Scientific or technical background required for work program

A background in applied mathematics and/or image analysis and/or artificial intelligence is needed. Programming skills (java and/or python) are also required.

Title of work program 19

Mechanisms of tau and syn spreading related to Alzheimer's and Parkinson's diseases progression

Description of the work program

Neurodegenerative diseases are the fourth leading cause of death in France and more than 900,000 patients suffered from Alzheimer's disease in 2018 with a steadily progressing incidence. Like Alzheimer's, also Parkinson's disease (PD) prevalence is steadily increasing. Indeed, the failure of clinical trials on drug candidates to treat AD and PD diseases in the last 10 years and more, is flagrant. One of the major reasons for this, is the lack of knowledge of the molecular mechanisms that lead to neurodegeneration both in AD and PD patients, although our knowledge of the disease has increased in recent years. Briefly, AD is neurodegenerative disorder characterized by extracellular accumulation of amyloid-β and intracellular formation of hyperphosphorylated tau protein inclusions. PD is marked by alpha-synuclein aggregates accumulation (Lewy bodies) in neurons, leading to the loss of dopaminergic cells. The propagation of pathological tau and synuclein proteins follows a prion-like mechanism, involving its seeding and transfer along connected neurons. However, important aspects of tau/syn biology remain still open questions: (i) the mechanisms involved in tau/syn transfer; (ii) which type of tau/syn assemblies (oligomers or fibrils) is mostly transferred and has the highest seeding activity; (iii) in which cell compartment tau seeding take place. Here, applying confocal microscopy and *in situ cryo-electron microscopy approaches*, we will make fundamental contributions to a mechanistic understanding of AD's and PD's diseases by investigating where tau and syn fibrillation takes place, how the amyloids protein spreads, and which assemblies are transferred.

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

Sartori-Rupp, A*. Cordero-Cervantes, D*. Pepe, A.* et al. Nat Commun 10, 342. 2019

Dilsizoglu Senol, A. Pepe A. et al. Sci Rep 9, 5741. 2019.

Grudina C et al., Neurobiol Dis 2019

Chastagner, et al. EMBO Molecular Medicine 12, e12025, 2020

Cordero, Zurzolo. EMBO J 40, e105789, 2021

Pepe, A. et al. Science Advances 8, eabo0171.2022

Chakraborty R et al., Cell Death Dis. 2023

Scientific or technical background required for work program

We are looking for a highly motivated candidate who has already demonstrated an excellent ability to carry out experiments during previous placements. A background in cell biology is required, as well as a good level of written and spoken English. Skills in cell culture, microscopy and/or image analysis and cryo-EM data analysis are welcome.

Title of work program 20

Characterization of involution-like mammary organoid model

Description of the work program

The ***mammary gland*** is a remarkably dynamic organ which primordial role is to produce milk to feed the progeny. This organ is able to undergo repeated estrus/reproductive cycles with profound tissue expansion and differentiation during pregnancy/lactation and regression/remodeling during involution. **Interestingly, we identify senescent cells during mammary gland involution.**

Cellular senescence is a form of stress response characterized by a stable cell cycle arrest, induced via activation of p53/p21 and p16INK4a/pRB pathways, and acquisition of a secretory phenotype, termed as senescence-associated secretory phenotypes (SASPs).
Senescence is associated with a variety of biological and pathological processes. Of note, senescence is important for embryonic development by facilitating the removal of transient structures and tissue remodeling

Organoids have emerged as a powerful new tool in many fields such as disease modeling, tissue organization, stem cell and developmental biology. In 2020, our laboratory developed a mammary organoids model mimicking lactation and involution processes. However, a deep characterization of this model is needed.

Therefore, this project aims to: a/ characterize senescent cells identity; b/ analyze the proportion of apoptotic vs senescent cells and their cell fate, and c/ identify cellular and molecular mechanisms that trigger epithelial cell death in involution-like organoids.

Tutor/supervisor

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

https://doi.org/10.3389/fcell.2020.00068
https://doi.org/10.21769/BioProtoc.3996

Scientific or technical background required for work program

Candidates with the following skills will be appreciated:

- Cell culture
- Immunofluorescence & imaging (histology, 3D imaging)
- Gene expression analysis (RNA extraction & RT-qPCR)

Title of work program 21

Investigating Mycobacterial Modulation of Nuclear Mechanodynamics in Macrophages

Description of the work program

CONTEXT

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb) and represents the tenth cause of death worldwide and a leading contributor to the antimicrobial resistance crisis[1]. One of the first lines of defense against Mtb are pulmonary macrophages, which patrol the lung microenvironments and play a dual role in pathogen containment on the one hand and as a survival niche on the other hand[2]. While macrophages in the alveolar lumen are more permissive towards Mtb, having an anti-inflammatory polarization state, interstitial macrophages are considered more restrictive, having a pro-inflammatory polarization state[3]. Importantly, the spectrum of polarization is associated with nuanced innate immune defenses, which also impact inflammation and tissue pathology. Mtb is able to manipulate and evade macrophages' innate defenses through effectors and virulence factors, which can exit the phagosome compartment and access the cytosol either by secretion or by vesicle-mediated delivery[4,5]. This enables the pathogen to target different host cell organelles, hijack immune pathways to modulate infection, subvert immune responses, and enhance its own survival within the macrophage niche. Some Mtb effectors were also shown to harbor nuclear localization sequences, enabling translocation to the host cell nucleus and modulation of host gene expression[6-8]. Interestingly, recent findings showed an inverse relationship between pro-inflammatory signals and the expression of nuclear envelope proteins, which act as mechano-regulators of macrophage polarization by modifying nuclear size and stiffness[9]. This suggests that physical changes in the nuclear structure could significantly affect the cellular function and ability to respond to invading pathogens. Here we hypothesize that differential induction of pro-inflammatory signals during Mtb phagocytosis could alter nuclear conformation, potentially impacting the immune response and pathogen control. Greater understanding of the mechanisms influencing macrophage polarization in relation to Mtb infection could provide insights for developing original weapons against this global infectious killer.

AIM

This project aims to examine the nuclear mechanodynamics in macrophages during mycobacterial infection, including the impact of Mtb-nuclear proximity on intracellular replication, macrophage polarization, and immune responses. Overall, this study will contribute to understanding the innate immune response to Mtb, its intracellular survival, and host modulation.

APPROACHES

Human THP-1 macrophages will be genetically engineered to express fluorescently labeled proteins associated with the nuclear envelope and mechanics. This will be achieved using the Sleeping Beauty transposon system and nucleofection. We will infect resting macrophages with a biosafety level 2 (BSL2) mycobacterial model organism, which constitutively expresses a different fluorescent marker. This will allow us to track the movement of mycobacteria and their localization within individual host cells. Using either microplates or a customized variant of our microfluidic Hexa-device[10], we will carry out short-term time-lapse imaging of early infection events. We will mostly focus on the localization and replication state of the bacilli relative to changes in nuclear size and shape over time and cytoskeletal remodeling upon infection. To further assess the host cell status in relation to the pathogen's subcellular localization, we will use live cell probes to quantify the oxidative state, pH, and viability of infected macrophages.

Finally, we will carry out quantitative single-cell analysis of host cells by segmenting and tracking nuclear and mycobacterial localization to understand the interplay between mycobacteria and macrophage nuclear dynamics and fate.

EXPECTED OUTCOMES AND SIGNIFICANCE

This project is expected to provide first insights into the impact of mycobacterial infection on host-cell nuclear envelope mechanics and the consequences for both host and pathogen during the early infection stage. Understanding these interactions could reveal new therapeutic targets by showing how nuclear physical remodeling affects macrophage responses to mycobacterial infection. Ultimately, this work may not only advance our understanding of TB pathogenesis but also pave the way for therapeutic interventions that disrupt pathogen-driven nuclear modulation in host cells.

LEARNING OPPORTUNITIES

In the Microbial Individuality and Infection Team, the Erasmus student will gain hands-on experience in molecular biology, genetic engineering of both eukaryotic and mycobacterial cells, advanced imaging techniques, and their broader applications in infection biology. They will experience an interdisciplinary environment, working at the interface of microbiology, cell biology, and microscopy, laying the foundation for a potential future PhD project in infection biology. This 6- to 12-month exploratory project will be carried out using a relevant BSL2 model mycobacterium and will allow us to develop the necessary tools and analytical pipeline to expand the study on the TB pathogen. Indeed, in a follow-up of the project, e.g., within a PhD program, the student could further investigate the molecular basis of Mtb-nuclear interactions, explore therapeutic targets, and assess their potential to enhance anti-TB drug efficacy using preclinical in vitro models.

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7. Yaseen I, Kaur P, Nandicoori VK, Khosla S. Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. **Nat Commun**. (**2015**) 6:8922. doi: 10.1038/ncomms9922. PMID: 26568365.

8. Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK, Khosla S. The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. **Nucleic Acids Res**. (**2015**) 43(8):3922-37. doi: 10.1093/nar/gkv261. PMID: 25824946.

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10. Manina G, Griego A, Singh LK, McKinney JD, Dhar N. Preexisting variation in DNA damage response predicts the fate of single mycobacteria under stress. **EMBO J**. (**2019**) 38(22):e101876. doi: 10.15252/embj.2019101876. PMID: 31583725.

Tutor / Supervisor

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

Mistretta M, Cimino M, Campagne P, Volant S, Kornobis E, Hebert O, Rochais C, Dallemagne P, Lecoutey C, Tisnerat C, Lepailleur A, Ayotte Y, LaPlante SR, Gangneux N, Záhorszká M, Korduláková J, Vichier-Guerre S, Bonhomme F, Pokorny L, Albert M, Tinevez JY, †**Manina G**. Dynamic microfluidic single-cell screening identifies pheno-tuning compounds to potentiate tuberculosis therapy. **Nat Commun.** (**2024**) 15(1):4175. doi: 10.1038/s41467-024-48269-2. PMID: 38755132.

Mistretta M, Gangneux N, †**Manina G**. Microfluidic dose-response platform to track the dynamics of drug response in single mycobacterial cells. **Sci Rep.** (**2022**) 12(1):19578. doi: 10.1038/s41598-022-24175-9. PMID: 36379978.

Griego A, Douché T, Gianetto QG, Matondo M, †**Manina G**. RNase E and HupB dynamics foster mycobacterial cell homeostasis and fitness. **iScience.** (**2022**) 25(5):104233. doi: 10.1016/j.isci.2022.104233. PMID: 35521527.

†***Manina G**, Dhar N. Single-Cell Analysis of Mycobacteria Using Microfluidics and Time-Lapse Microscopy. **Methods Mol Biol.** (**2021**) 2314:205-229. doi: 10.1007/978-1-0716-1460-0_8. PMID: 34235654.

†***Manina G**, Griego A, Singh LK, McKinney JD, Dhar N. Preexisting variation in DNA damage response predicts the fate of single mycobacteria under stress. **EMBO J.** (**2019**) 38(22):e101876. doi: 10.15252/embj.2019101876. Epub 2019 Oct 4. PMID: 31583725.

Manina G and Mistretta M. Multiplexable microfluidic culture chamber for imaging monolayer growth of single cells, published on **19.11.2020**. Patent number EP3969174; US20220195486 (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020229629&tab=PCTBIBLIO).

Scientific or technical background required for work program

The ideal candidate is expected to have a solid background in genetics, microbiology, and cell biology, with experience working under sterile conditions. Proficiency in fluorescence microscopy and knowledge of bioimage analysis tools would be a plus. The student must also have a good command of both written and spoken English, work well in a team, and prove scientific curiosity and dedication to research.

Title of work program 22

Morphodynamic modelling of hematopoietic stem cells

Description of the work program

Biological context.

In vertebrates, hematopoietic stem cells are generated during the embryonic period. This occurs in arterial vascular tissue, particularly in the dorsal aorta, where specific (endothelial) cells undergo a morphological evolution that accompanies their extrusion from the aortic wall.

This emergence process, known as endothelial-hematopoietic transition (EHT), is characterized by a highly unusual curvature of the cell membrane towards the subaortic space. The cells are then released into the bloodstream and become hematopoietic stem cells, the origin of embryonic and adult blood and immune cells.

This type of cell emergence process has been studied qualitatively in zebrafish embryos in recent work [1, 2] using time-lapse confocal imaging. A recent algorithm [3], developed by our team, reconstructs confocal volumes to visualize membrane shape in 3D over time. The next step is to establish a model of membrane evolution to explain its morphological changes and quantify the associated biophysical parameters.

Project.

The internship's objective is to define a shape regression model [4] for the evolution related to EHT. The first approach is to study regression models based on the Helfrich energy, which is widely used to study membrane equilibrium. This functional, which depends on membrane curvatures, models the elastic deformation of the membrane under mechanical stress and enables its geometric deformation to be described.

Subsequently, quantitative methods need to be developed to estimate the physical parameters involved in the EHT process (e.g. tissue forces, blood flow pressure). Their estimation can be achieved via an inverse problem approach based on analytical models [5] or using a machine learning approach [6].

Beyond EHT modeling, this project aims to explore new frameworks for shape evolution, which is a vast field of study for several biomedical applications [7].

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Scientific or technical background required for work program

Expected work. A good knowledge of mathematics and machine learning theory is needed for this topic. After a review of the literature on shape regression models and Helfrich energy, a novel method for EHT analysis will be developed. The results will be validated on confocal time-lapse sequences in collaboration with biologists.

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

[1] L. Torcq, S. Majello, C. Vivier, and A. A. Schmidt, “Tuning apico-basal polarity and junctional recycling in the hemogenic endothelium orchestrates pre-hematopoietic stem cell emergence complexity,” *eLife*, vol. 12, 2023.
[2] M. Lancino, S. Majello, S. Herbert, F. De Chaumont, J.-Y. Tinevez, J.-C. Olivo-Marin, P. Her- bomel, and A. Schmidt, “Anisotropic organization of circumferential actomyosin characterizes hematopoietic stem cells emergence in the zebrafish,” *Elife*, vol. 7, p. e37355, 2018.
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[4] S. Durrleman, X. Pennec, A. Trouvé, G. Gerig, and N. Ayache, “Spatiotemporal atlas estimation for developmental delay detection in longitudinal datasets,” in *Medical Image Computing and Computer-Assisted Intervention–MICCAI 2009: 12th International Conference, London, UK, September 20-24, 2009, Proceedings, Part I 12*, pp. 297–304, Springer, 2009.
[5] 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,” *Scientific Reports*, vol. 7, no. 1, p. 9178, 2017.
[6] S. A. Malingen and P. Rangamani, “Modelling membrane curvature generation using mechan- ics and machine learning,” *Journal of the Royal Society Interface*, vol. 19, no. 194, p. 20220448, 2022.
[7] R. Sarkar, D. Darby, S. Meilhac, and J.-C. Olivo-Marin, “3D cell morphology detection by asso- ciation for embryo heart morphogenesis,” *Biological Imaging*, vol. 2, p. e2, 2022.

Title of work program 23

Motion switching along cells receptors trajectories

Description of the work program

Motion classification for cells receptors.

Nowadays, fluorescence microscopy coupled with tracking algorithms allows the detection of particles at the cell membrane and the reconstruction of their trajectories. In a recent paper [1], these techniques are used to describe the trajectories of specific receptors (CCR5) in human cells that are involved in several inflammatory processes and in HIV infection. Dynamic characterization makes it possible to identify several receptor populations and study their role in the infection process.

The article [1] establishes a statistical method to distinguish standard classes of motion for CCR5 receptors (sub-diffusive, Brownian, directed). The method uses a decision test for characterizing motion [2] based on the maximum distance from the initial position.

This method has been improved by recent internship work, defining a machine learning method for classifying a larger family of movements. This work is based on the geometric characteristics of the trajectories [3] enabling different subdiffusive behaviors to be distinguished (Continuous-Time Random Walk, Ornstein-Uhlenbeck, Fractional Brownian Motion).

Project.

The methods presented above allow us to classify movements for trajectories of a given length, thus describing their overall dynamics. However, the movement of receptors can change along their trajectory, depending on their biological environment and fate. The detection of switching points provides relevant information on receptor interactions and the effect of different treatments on them. There is little work on the detection of switching points, and existing approaches develop statistical methods based on the properties of Brownian motion [4].

The aim of this internship is to develop a new method for analyzing changes in motion along trajectories. The main objective is to define suitable parameters whose variation over time corresponds to a change in motion. In particular, the method should highlight motion variations in the sub-diffusive regime, which mainly characterize the behavior of CCR5 receptors.

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Scientific or technical background required for work program

Expected work. A good knowledge in mathematics and machine learning theory is need for this topic. After a review of the literature on stochastic processes and relative methods for motion classification, a new method of motion switching will be developed. The method validation will be performed on simulated trajectories and on experimental data.

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

[1] F. Momboisse, G. Nardi, P. Colin, M. Hery, N. Cordeiro, S. Blachier, O. Schwartz, F. Arenzana-Seisdedos, N. Sauvonnet, J.-C. Olivo-Marin, *et al.*, "Tracking receptor motions at the plasma membrane reveals distinct effects of ligands on ccr5 dynamics depending on its dimerization status," *Elife*, vol. 11, p. e76281, 2022.

[2] V. Briane, C. Kervrann, and M. Vimond, "Statistical analysis of particle trajectories in living cells," *Physical Review E*, vol. 97, no. 6, p. 062121, 2018.

[3] Y. Meroz and I. Sokolov, "A toolbox for determining subdiffusive mechanisms," *Physics Reports*, vol. 573, pp. 1–29, 2015.

[4] V. Briane, M. Vimond, C. Valades-Cruz, A. Salomon, C. Wunder, and C. Kervrann, "A sequential algorithm to detect diffusion switching along intracellular particle trajectories," *Bioinformatics*, vol. 36, no. 1, pp. 317–329, 2020.

Title of work program 24

HprK-dependent phosphorylation of HPr and its role in the interaction of *Neisseria menigitidis* to target cells

Description of the work program

Infection of host cells by pathogens usually requires the synthesis of specific proteins directly related to virulence, which can be triggered by specific signals including the contact with host cells. Signals arising from changes in carbon source availability seem to be transmitted via the phosphorylation state of the carbohydrate **p**hospho**t**ransferase **s**ystem (PTS) proteins.

N. meningitidis is commensal of the nasopharynx where the first colonization occurs asymptomatically (10 to 15% of the general population are asymptomatic carriers), but in some circumstances, highly pathogenic lineages provoke severe and fatal infections dominated by septicaemia and meningitis. *N. meningitidis* may therefore survive and multiply in several anatomic sites where it is submitted to variations in the carbon sources. Several genes, which were found to be essential for invasive infection by *N. meningitidis*, were related to carbon metabolism, including the phosphotransferase system (PTS). Indeed, we have previously shown that HPr protein of *N. meningitidis*, a major component of the PTS system, plays an important role in the virulence of meningococci. This protein interacts with the LysR-type transcriptional regulator, CrgA and modulates the interaction of *N. meningitidis* to epithelial cells and survival during dissemination of the bacteria. On the other hand, HPr is the target of the bifunctional HPr kinase/phosphorylase (HprK) that catalyses the phosphorylation and dephosphorylation of the residue Ser^{46} in the protein substrate HPr. We therefore hypothesize that the phosphorylation state of HPr may impact the interaction of *N. meningitides* to epithelial cells. This project aims therefore to explore the role of HprK-dependent phosphorylation of HPr on the interaction of *N. meningitides* to target cells and the expression of virulence factor factors involved in this interaction.

This project will be performed within the Invasive Bacterial Infections Unit (IBI). 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. Several molecular and cell biology approaches will be also involved.

Tutor/supervisor

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

- Derkaoui M, Antunes A, Abdallah JN, Poncet S, Maze A, et al. (2016) Transport and Catabolism of Carbohydrates by Neisseria meningitidis. Journal of Molecular Microbiology and Biotechnology 26: 320-332.
- Derkaoui M, Antunes A, Poncet S, Abdallah JN, Joyet P, et al. (2016) The phosphocarrier protein HPr of Neisseria meningitidis interacts with the transcription regulator CrgA and its deletion affects capsule production, cell adhesion and virulence. Mol Microbiol.
- Antunes A, Derkaoui M, Terrade A, Denizon M, Deghmane AE, et al. (2016) The Phosphocarrier Protein HPr Contributes to Meningococcal Survival during Infection. PLoS ONE 11: e0162434.
- Deghmane AE, Giorgini D, Maigre L, Taha MK (2004) Analysis in vitro and in vivo of the transcriptional regulator CrgA of Neisseria meningitidis upon contact with target cells. Mol Microbiol 53: 917-927.

Scientific or technical background required for work program

Using *Neisseria meningitidis* as a model, the trainee will learn how a pathogen might manipulate host-cell signalling at the interface of host-pathogen interaction. The trainee is expected to have technical skill in conventional bacteriology (bacterial culture, isolation of bacterial, storage of bacterial cultures), molecular and cellular biology (recombinant DNA manipulation, immunoblot, cell culture). The trainee is also expected to communicate frequently in the lab meetings to present the progress of his work and discuss the difficulties he encountered to find out alternative solutions.