Erasmus+

INSTITUT PASTEUR

Projects 2020-2021

Erasmus+

Institut Pasteur 2020-2021

RESEARCH CENTRE

Legal name: **Institut Pasteur**

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Brief description of your Institution

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

Description of the work program(s)

See projects on following pages

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

The laboratories at Pasteur have proposed 32 projects for Erasmus internships (see following pages). Students may also contact other laboratories at Pasteur to apply for an internship, even if the laboratories have not presented a project.

FACILITIES (not compulsory for the host centre)

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

- **Additional salary** (some centres offer an additional salary ranging from 200 to 1000 €/month) **X** YES ☐ NO
  additional salary of approximately 570 euros/month is paid by the host lab (3.90 euros/hour, 7 hours/working day)

Title of the work program 1

Senses4All: from hearing and/or balance disease mechanisms to adapted gene therapies

Description of the work program

Communication is the essence of social interaction; untreated declines in **hearing and balance** abilities, whether of genetic origin or due to aging and/or environment, often results in social isolation, depression and reduced physical and cognitive function. Hearing impairment is the most frequent cause of inherited sensory deficits in humans, and the number of hearing-impaired patients dramatically increases with aging, especially in loud environments. Unfortunately, we make noise (including leisure music) to muffle noise, without paying attention to the hazardous effects of sound on our hearing. The prevalence of hearing impairments (often associated with balance deficits) will increase from > 460 million in 2017 to over 1 billion individuals by 2050 (http://www.who.int). These major **sensory deficits** still represent unmet medical needs, since treatment options are largely missing.

Despite their high prevalence, inherited or acquired progressive **hearing disorders** have not been well studied or understood; resulting in many important unanswered questions: how do our inner ear sensory organs ensure long-term normal function? what are the causal genes & related pathways involved in hearing and balance maintenance? how do external factors and age impacts these senses? & what are the treatment solutions?

The project is interdisciplinary, with cutting-edge techniques in “Omics”, biochemical and computational analyses, imaging, cell biology, physiology (some benefiting from external collaborations), and gene therapy tools (**gene supplementation** & **CRISPR/Cas9 gene editing**) designed to enable a thorough and accurate phenotyping and treatment of available defective mutant mice.

This project includes approaches to:

1- identify molecular mechanisms underlying function of deafness defective proteins by investigating their **properties through integrative biological interaction networks**.

2- **determine where, when and how inner ear abnormalities manifest** in the available defective mice to elucidate the precise molecular and cellular mechanisms underlying their hearing and balance sensory deficits (disease signature).

3- Decipher if (& how) external factors such as **exposure to intense sound impacts** the onset, progression and/or severity of the disease.

4- Evaluate **gene therapy** (gene replacement & gene editing via CRISPR/Cas9) efficacy to restore normal **sensory modalities** in available defective mice.

Tutor/supervisor

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

Delmaghani S* and **El-Amraoui, A* (2020)** The inner ear gene therapies take off: current promises and future challenges. ***J. Clin. Medicine*,** 9, 2309; doi:10.3390/jcm9072309. *Co-senior authorships. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7408650/

Geleoc G*****. **El-Amraoui A***. Disease mechanisms and gene therapy for Usher syndrome. *Hear. Res.* 4:107932 **(2020). (**https://www.sciencedirect.com/science/article/pii/S0378595519304733**)**

Dunbar L, Patni P, Aguilar C, Mburu P, Corns L, Wells H, Delmaghani S, .., Brown S, Marcotti W, **El-Amraoui A***, Bowl M* **(2019)** Clarin-2 is essential for hearing by maintaining stereocilia integrity and function. ***EMBO Mol. Med.*** 11(9):e10288. doi: 10.15252/emmm.201910288. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6728604/

Dulon D*, Papal S, Patni P, Cortese M, Vincent P, Tertrais M, Emptoz A, Tlili [A,] Bouleau Y, Michel V, Delmaghani D, Aghaie A, Pepermans E, Allegria-Prevot O, Akil O, Lustig L, Avan P, Safieddine S, Petit C*, and **El-Amraoui A***. **(2018)** Clarin-1 defect results in a rescuable auditory hair cell synaptopathy. ***J. Clin. Invest.*** 128(8):3382-3401. doi:10.1172/JCI94351. https://www.jci.org/articles/view/94351

Michel V, Booth K, Patni P, Cortese M, Azaiez H, Bahloul A, Kahrizi K, Labbé M, Emptoz A, Lelli A, Dégardin J, Dupont T, Aghaie A, Oficyalska D, Picaud S, Najmabadi H, Smith RJ, Bowl MR, Brown SDM, Avan P, Petit C, **El-Amraoui A* (2017)** CIB2, defective is isolated deafness, is key to auditory hair cells mechanotransductioin and survival. ***EMBO Mol. Med.*** 9:1711-1731. http://embomolmed.embopress.org/content/9/12/1711.long

Cortese M., Papal S., Pisciottano F., Elgoyhen A.B., Hardelin J.-P., Petit C., Franchini L.F*, & **El-Amraoui A*. (2017)** Spectrin βV adaptive mutations and changes in subcellular location correlate with emergence of hair cell electromotility in mammalians. ***Proc. Natl Acad. Sci. USA*.** 114(8):2054-2059. doi: 10.1073/pnas.1618778114. http://www.pnas.org/content/114/8/2054.long

Schietroma S., Parain K., Estivalet A., Aghaie A., Boutet de Monvel J., Picaud S. Sahel J-A. Perron M., **El-Amraoui A*** & Petit C*. **(2017)** Shaping of the photoreceptor outer segment by the calyceal processes of the inner segment. ***J. Cell Biol.*** 216, 1849-1864. *Co-senior and corresponding authors. F1000 F1000 Medicine "Recommended" selection. http://jcb.rupress.org/content/216/6/1849.long

Lelli A, Michel V, Boutet de Monvel J, Cortese M, Bosh-Grau M, Aghaie A, Perfettini I, Dupont T, Avan P, **El-Amraoui A***, Petit C***. (2016)** Class III myosins shape the auditory hair bundles by limiting microvilli and stereocilia growth. ***J. Cell Biol.*** 212, 231-44. *Co-senior and corresponding authors. F1000 F1000"Recommended" selection; # cover. http://jcb.rupress.org/content/212/2/231.long

Kamiya K, Michel V, Giraudet F, Riederer B, Foucher I, Papal S, Perfettini I, Le Gal S, Verpy E, Xia W, Seidler U, Georgescu MM, Avan P*****, **El-Amraoui A***, Petit C***. (2014)** An unusually powerful mode of low-frequency sound interference due to defective hair bundles of the auditory outer hair cells. ***Proc Natl Acad Sci USA.*** 111: 9307-9312. *Co-senior and corresponding authors. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4078795/

Sahly, I., Dufour, E., Schietroma, C., Michel, V., Bahloul, A., Perfettini, I., Pepermans, E., Estivalet, A., Carette, D., Aghaie, A. Ebermann I, Lelli A, Iribarne M, Hardelin JP, Weil D, Sahel, J-A, **El-Amraoui, A*.** and Petit, C*. **(2012)** Localization of Usher 1 proteins to the photoreceptor calyceal processes,

which are absent from mice. ***J. Cell Biol.*, 199**, 381-399. *Corresponding authors. F1000 F1000 Medicine “Recommended” selection. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3471240/

Scientific or technical background required for work program

There are different aspects to the project, from *in silico* bioinformatic analyses with comparisons to other already available home-made “Omics” data, to in-depth physiological-, morpho-, and molecular-phenotyping of the mutant mice. **Motivated candidates with interests and skills covering above-mentionned approaches will be considered.**

Title of the work program 2

Role of dynamin-like protein HP0963 in *Helicobacter pylori*

Description of the work program

Bacteria come in different shapes, varying from rod (bacilli) to spheres (cocci) to helical and spirals (spirochetes) among many others, and undergo changes in cell shape via a dynamic process that is influenced by their environment. We have shown that *Helicobacter pylori* undergoes a morphological transition from helical-rod to coccoid as a mechanism to escape the innate immune system and that maintenance of the helical shape is essential for survival in its unique niche, the stomach. Thus, cell shape plays an important role in infection, and host cells directly influence bacterial cell shape. *H. pylori* is a microaerophilic ε-proteobacterium that is the major cause of peptic ulcers and gastric cancer. Its cell shape together with flagellar motility, its capacity to adhere to host cells and the secretion of virulence factors CagA and VacA are essential for colonization of the stomach.

In this project we will study the role of the uncharacterized gene HP0963 in cell shape in *H. pylori*. The gene was identified as a cell-shape determinant in a screen for cell shape mutants using an ordered mini*Tn*3 transposon library. All 1590 *H. pylori* genes were cloned individually on plasmids in *E. coli* and interrupted by insertion of the mini*Tn*3 transposon that contains a kanamycin-resistance cassette. A wild type *H. pylori* strain was transformed with the mini*Tn*3 library and transformants were analyzed by phase contrast microscopy and flow cytometry. Cells in which HP0963 is interrupted have a filamentous morphology, suggesting a defect in cell division. HP0963 encodes a protein with similarities to eukaryotic dynamin, a GTP-ase involved in budding of vesicles, fusion of membranes and cytokinesis. HP0963 has a predicted dynamin domain containing a GTP-binding motif, a coiled-coil domain, and potentially interacts with the membrane through a lipid-binding domain. To understand the function of HP0963 in *H. pylori*, we will construct a deletion mutant of HP0963 and analyze *in vitro* growth and viability, and analyze its morphology by phase contrast microscopy, determine its cellular localization by fluorescence microscopy and characterize functional domains of the protein by complementation studies.

Tutor/supervisor

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

Veyrier FJ, N Biais, P Morales, N Belkacem, C Guilhen, S Ranjeva, O Sismeiro, G Péhau-Arnaudet, EP Rocha, C Werts, M-K Taha, IG Boneca. (**2015**) Common cell shape evolution of two nasopharyngeal pathogens**. *PLoS Genet*.** 11(7):e1005338.

Gélis-Jeanvoine S, S Lory, J Oberto, N Buddelmeijer. (**2015**) Residues located on membrane-embedded

flexible loops are essential for the second step of the apolipoprotein N-acyltransferase reaction. ***Mol Microbiol***. 95: 692-705.

Chaput C, C Ecobichon, N Pouradier, JC Rousselle, A Namane, IG Boneca. (**2016**) Role of the N-acetylmuramoyl-l-alanine amidase, AmiA, of *Helicobacter pylori* in peptidoglycan metabolism, cell daughter separation and virulence. ***Microb Drug Resist***. 22(6):477-86.

Contreras-Martel C, A Martins, C Ecobichon, D Maragno Trindade, P-J Matteï, S Hicham, P Hardouin, M El Ghachi, IG Boneca*, A Dessen*. (**2017**) Molecular architecture of PBP2:MreC core bacterial cell wall synthesis complex. ***Nat Commun***. 8(1):776.

Nozeret K, A Boucharlat, F Agou, N Buddelmeijer. (**2019**) A sensitive fluorescence-based assay to monitor enzymatic activity of the essential integral membrane protein Apolipoprotein N-acyltransferase. ***Sci Rep***. 9:15978.

Scientific or technical background required for work program

We are looking for a motivated student to take on this project where bacterial genetics and biochemical approaches are combined. Some hands-on lab experience is a plus.

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Title of the work program 3

Investigating the multidimensional susceptibility to chronic inflammatory diseases using mice of the collaborative cross

Description of the work program

Complex chronic diseases are caused by the accumulation of genetic, microbial and lifestyle factors. The number and complexity of such factors makes prediction of pathogenesis and therapy particularly difficult. Although a single factor is rarely sufficient to trigger pathology, genetic and environmental factors have so far been studied in isolation. Nevertheless, a substantial number of genetic variants have been associated with disease risk and the concomitant lifestyle shift and excessive hygiene are thought to contribute to the increased incidence in inflammatory diseases in industrialized countries. We recently reported that antibiotics-driven depletion of microbiota during early life increases the susceptibility to allergy and experimental colitis later in life. In order to explore the gene x environment interactions underlying vulnerability to inflammatory pathology, we propose to use mice of the Collaborative Cross (CC), that model human genetic diversity, exposed or not to antibiotics early in life. We expect spontaneous inflammation in a small set of CC strains while other strains may display resistance to disease even upon environmental challenge. Our study will allow the identification of new gene-environment associations responsible for the establishment of chronic inflammatory diseases and will generate multiple biomarkers of vulnerability as well as potential targets for prevention and treatment of chronic inflammatory diseases. In particular, we focus on type 1 diabetes and inflammatory bowel diseases as examples of autoimmune and autoinflammatory diseases, respectively.

Tutor/supervisor

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

- Ziad Al Nabhani, Gérard Eberl, Imprinting of the immune system by the microbiota early in life, Mucosal Immunol (2020). https://doi.org/10.1038/s41385-020-0257-y.

- Ziad Al Nabhani, Sophie Dulauroy, Emelyne Lécuyer, Bernadette Polomack, Pascal Campagne, Marion Berard & Gérard Eberl, Excess calorie intake early in life increases susceptibility to colitis in adulthood, Nat Metab (2019) doi:10.1038/s42255-019-0129-5.

- Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, Déjardin F, Sparwasser T, Bérard M, Cerf-Bensussan N, Eberl G, A Weaning Reaction to Microbiota Is Required for Resistance to Immunopathologies in the Adult, Immunity 2019 05;50(5):1276-1288.e5.

- Rincel M, Aubert P, Chevalier J, Grohard PA, Basso L, Monchaux de Oliveira C, Helbling JC, Lévy É, Chevalier G, Leboyer M, Eberl G, Layé S, Capuron L, Vergnolle N, Neunlist M, Boudin H, Lepage P, Darnaudéry M, Multi-hit early life adversity affects gut microbiota, brain and behavior in a sex-dependent manner, Brain Behav. Immun. 2019 Aug;80:179-192.

Scientific or technical background required for work program

The current project is based on the long-term screening of over 250 mice of different genotypes and of both sexes and requires *in vivo* experimentation including breeding, blood sampling, collection of feces, i.p. and s.c. injections as well as organ dissection (including pancreas, gut, liver and fat pads). Histological analysis and pathological scoring of pancreata are carried out using the imageJ software. In addition, molecular targets in the blood (cytokines, metabolic hormones and corticosterone) are quantified by classic ELISA and highly sensitive multiplex techniques. Analyses are carried out with big datasets using various statistical methods including multifactorial ANOVAs and PCAs. Moreover, quantitative trait locus (QTL) analysis will be used to link specific genomic regions to phenotypes of interest in order to unravel new genes of disease susceptibility.
Previous experience with mice is highly recommended.

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Title of the work program 4

Dissecting the role and function of type III interferons (IFNλ)

Description of the work program

Immune responses are highly variable from one individual to another with this variance due to intrinsic (age, sex); genetic and environmental factors[1]. Cytokines, which are the main instigators of immune responses, also show great diversity which enables host immune responses against diverse pathogens such as viruses, bacteria, and fungi. Type III interferons (IFN-lambdas(λ)) are important cytokines that inhibit viruses and modulate immune responses mainly in mucosal and epithelial sites of infection [2]. In addition 4 subtypes of IFNλ (1-4) have been identified which show high levels of genetic variability suggesting evolutionary selection. They have also recently been implicated in host immune responses to infection with SARS-CoV-2 [3]. However little is known about potential differences between the 4 subtypes in terms of their function, specificity or responsiveness to different microbes. This project will study and better characterize the different roles of type III interferons. This will involve cell culture of human cell lines and primary tissues (blood and tonsils). Multi-parameter flow cytometry will be utilized to characterize cell surface receptor expression levels on different cell types and in different culture conditions (eg with or without external stimulation). Digital ELISA assays will be developed that enable quantification of IFNλ subtypes from both cell cultures and clinical samples [4]. Depending on the results generated we may apply our findings to better understand the role of the different IFNλ subtypes in clinical situations such as acute (eg Covid-19, Dengue) or chronic (eg HCV, HIV) viral infection. In summary this project will provide a better understanding of the variability within type III interferon responses at the cellular and proteomic level, and help understand differences in immune responses to viral infection.

1. Duffy, D. Understanding immune variation for improved translational medicine. *Curr. Opin. Immunol.* **65**, 83–88 (2020).
2. Broggi, A., Granucci, F. & Zanoni, I. Type III interferons: Balancing tissue tolerance and resistance to pathogen invasionType III interferons in host–pathogen interaction. *J. Exp. Med.* **217**, (2020).
3. Park, A. & Iwasaki, A. Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host Microbe* **27**, 870–878 (2020).
4. Rodero, M. P. *et al.* Detection of interferon alpha protein reveals differential levels and cellular sources in disease. *J. Exp. Med.* **214**, 1547–1555 (2017).

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

Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients.

Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, Péré H, Charbit B, Bondet V, Chenevier-Gobeaux C, Breillat P, Carlier N, Gauzit R, Morbieu C, Pène F, Marin N, Roche N, Szwebel TA, Merkling SH, Treluyer JM, Veyer D, Mouthon L, Blanc C, Tharaux PL, Rozenberg F, Fischer A, **Duffy D*,** Rieux-Laucat F*, Kernéis S*, Terrier B*.
Science. 2020 Aug 7;369(6504):718-724. doi: 10.1126/science.abc6027. Epub 2020 Jul 13.

Plasma Type I IFN Protein Concentrations in Human Tuberculosis.
Llibre A, Bilek N, Bondet V, Darboe F, Mbandi SK, Penn-Nicholson A, Hatherill M, Rozenberg F, Scriba TJ, **Duffy D**.
Front Cell Infect Microbiol. 2019 Aug 22;9:296. doi: 10.3389/fcimb.2019.00296.

Mitochondrial double-stranded RNA triggers antiviral signalling in humans.
Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rötig A, Crow YJ, Rice GI, **Duffy D**, Tamby C, Nojima T, Munnich A, Schiff M, de Almeida CR, Rehwinkel J, Dziembowski A, Szczesny RJ, Proudfoot NJ.
Nature. 2018 Aug;560(7717):238-242

Distinctive roles of age, sex, and genetics in shaping transcriptional variation of human immune responses to microbial challenges.
Piasecka B*, **Duffy D***, Urrutia A, Quach H, Patin E, Posseme C, Bergstedt J, Charbit B, Rouilly V, MacPherson C R, Hasan M, Albaud B, Gentien D, Fellay J, Albert M L*, Quintana-Murci L*, Milieu Intérieur Consortium
PNAS 2018 Jan 16;115(3)

Detection of interferon alpha protein reveals differential levels and cellular sources in disease.
Rodero MP, Decalf J, Bondet V, Hunt D, Rice GI, Werneke S, McGlasson SL, Alyanakian MA, Bader-Meunier B, Barnerias C, Bellon N, Belot A, Bodemer C, Briggs TA, Desguerre I, Frémond ML, Hully M, van den Maagdenberg AMJM, Melki I, Meyts I, Musset L, Pelzer N, Quartier P, Terwindt GM, Wardlaw J, Wiseman S, Rieux-Laucat F, Rose Y, Neven B, Hertel C, Hayday A, Albert ML, Rozenberg F, Crow YJ*, **Duffy D***.
J Exp Med. 2017 May 1;214(5):1547-1555. doi: 10.1084/jem.20161451. Epub 2017 Apr 18.

Functional analysis using standardized whole blood stimulation systems defines the boundaries of a healthy immune response to complex stimuli.
Duffy D*, Rouilly V*, Libri V*, Hasan M, Beitz B, David M, Urrutia A, Bisiaux A, La Brie S, Dubois A, Delval C, Thomas S, Rogge L, Schmolz M, Quintana-Murci L, Albert M* for The Milieu Intérieur Consortium.
Immunity 2014 March 20 40(3):436-50

Scientific or technical background required for work program

Immunology, Cell culture, flow cytometry, ELISA, data analysis

Title of the work program 5

Analysis of flagellar composition of the spirochete Leptospira

Description of the work program

Motility is a key virulence determinant for spirochetes, which include the agents of leptospirosis (Leptospira interrogans), syphilis (Treponema pallidum) and Lyme disease (Borrelia burgdorferi). Spirochetes have spiral-shaped cells and they evolved characteristically powerful swimming capabilities that enable them to rapidly disseminate through connective tissue, blood, and all organs. The motility of spirochetes is unique in that it is conferred by flagella inserted at both poles of the cells and extended within the periplasm (endoflagella) between the outer and inner membranes. We propose to characterize the components of the endoflagellum and the chemotaxis signaling pathway and determine how these features facilitate *Leptospira* motility and host dissemination. A preliminary proteomic analysis of purified filaments from *Leptospira spp.* identified new putative flagellar-associated proteins. In this program, we will generate mutants of any gene encoding flagellar-associated proteins by using the allelic exchange and dcas9 approaches. We will test if deletion of each protein has an effect on cell morphology and motility (measurement of velocity and reversals in different viscous media), and filament geometry/morphology.
We will identify key determinants of the endoflagellar apparatus and chemotaxis pathways, including new flagellar or flagellar-associated proteins. Characterizing the molecular determinants of the spirochete flagella and defining the mechanisms by which these bacteria move, shall uncover paradigm-shifting mechanisms of bacterial motility. *Leptospira* shall serve as a new paradigm for bacterial flagella and as an instrumental model to understand spirochete motility with high impact for serious public health issues including syphilis and Lyme disease

Tutor/supervisor

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

- An asymmetric sheath controls flagellar supercoiling and motility in the leptospira spirochete. Gibson KH, Trajtenberg F, Wunder EA, Brady MR, San Martin F, Mechaly A, Shang Z, Liu J, Picardeau M, Ko A, Buschiazzo A, Sindelar CV. Elife. 2020 Mar 11;9:e53672.
- Gene silencing based on RNA-guided catalytically inactive Cas9 (dCas9): a new tool for genetic engineering in Leptospira. Fernandes LGV, Guaman LP, Vasconcellos SA, Heinemann MB, Picardeau M, Nascimento ALTO. Sci Rep. 2019 Feb 12;9(1):1839.
- FcpB Is a Surface Filament Protein of the Endoflagellum Required for the Motility of the Spirochete Leptospira. Wunder EA Jr, Slamti L, Suwondo DN, Gibson KH, Shang Z, Sindelar CV, Trajtenberg F, Buschiazzo A, Ko AI, Picardeau M. Front Cell Infect Microbiol. 2018 May 8;8:130.

Scientific or technical background required for work program

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

Title of the work program 6

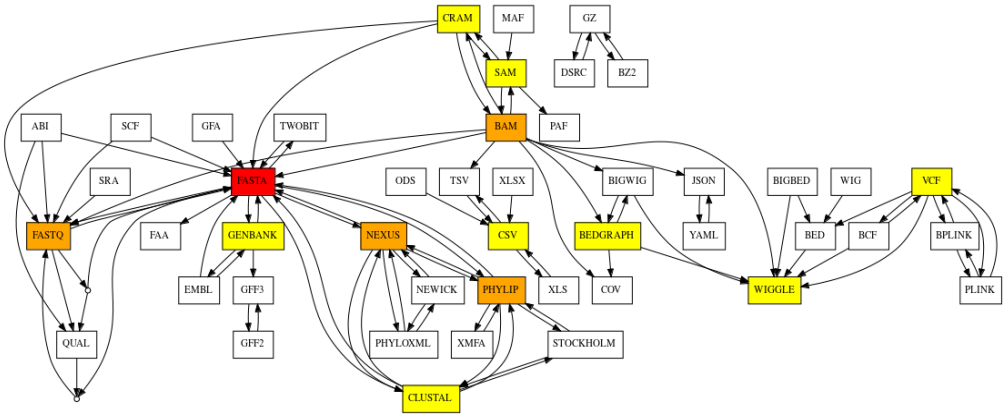
Web interface for the bioconvert project, a universal format converter for life sciences

Description of the work program

Life science uses many different formats. They may be old, or with complex syntax and converting those formats may be a challenge. Bioconvert project aims at providing a common tool / interface to convert life science data formats from one to another as a command line but also as a web application.

Many conversion tools already exist but they may be dispersed, focused on few specific formats, difficult to install, or not optimised. With Bioconvert, we plan to cover a wide spectrum of format conversions; we will re-use existing tools when possible and provide facilities to compare different conversion tools or methods via benchmarking. New implementations are provided when considered better than existing ones.

We currently have 50 formats and 100 conversions (see image below) and the tool has reached a stable version. Our goal is now to provide a web application that can be deployed easily. An instance will be deployed on our server (Institut Pasteur) as a demonstration. More importantly the web application should be made standard and open so that anyone can install an instance of the server locally. A secondary goal is to communicate via a research paper on the bioconvert tool and the web apllication itself.



Tutor/supervisor

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

- Sequanix : a dynalic graphical interface for snakamake workflows. D. Desvillechabrol, R, Legendre, T, Cokelaer. Bioinformatics 34 (11), 1934-1936 (2018)
- Sequana : a set of Snakemake NGS pipelines. T. Cokelaer ; D. Desvillechabrol, R, Legendre, M, Cardon. Journal of Open Software 2 (16) , 352 (2017).
- GDSCTools for mining pharmacogenomic interactions in cancer. T. Cokelaer, E. Chen, F Iorio, MP Menden, Lightfoot H, J Saez-Rodriguez, Bioinformatics 34 (7), 1226-1228. (2017).
- A landscape of pharmacogenomic interactions in cancer. F Iorio et al, Cell 166 (3), 740-754 (2016).

Scientific or technical background required for work program

Bioconvert tries to use as many existing bioinformatics tools as possible. Those tools are part of the bioinformatics ecosystem and may be written in many different languages such as Perl, C, fortran, R, Python, etc. Nevertheless, the candidates do not need to known all those languages. Since Bioconvert is primarily developped in Python the candidate should at least be a very good programmer in Python. A good knowledge of Unix environment is also essential to the task. The main task is the deployment of a web application. Therefore we expect to the candidate to have a good understanding of existing web framework. The application should be based on Django or flask, which are also Python-framework. A draft version of the web application exists in Flask but the candidate will have all freedom to redesign the web application if needed. We also expect the candidate to beneficiate from a local infrastructure based on Kubernetes / Docker to facilitate the deployment of the application. Finally, the candidate will be helped in his comprehension of Bioconvert by at least 2 senior bioinformaticians and the Kubernetes infrastruture is already in place.

Title of the work program 7

Discover the principles of soma-to-germline RNA transfer and its biological functions

Description of the work program

Heritable traits are predominantly encoded within genomic DNA, but it is now appreciated that epigenetic information is also inherited through DNA methylation, histone modifications and RNA.
The capacity of RNA to alter trait inheritance became evident with the discovery of RNA interference (RNAi) over 20 years ago. Pioneering experiments by Mello and Fire showed that *C. elegans* treated with double-stranded RNAs (dsRNAs) displayed gene silencing that persisted in descendants not originally exposed to the dsRNAs. Interestingly, heritable RNAi response in worms was induced not only by the direct injection of dsRNA into their germline but also by the accumulation of dsRNA in intestinal cells from ingested bacteria. These observations suggest that small RNAs in somatic cells can be transferred to germ cells to provide heritable epigenetic information of acquired traits.

Recent observations in *C. elegans* have shown that somatic small RNAs can actually control the transgenerational inheritance of learned behaviors, a trait that is acquired through the soma. However, the underlying mechanisms, such as whether the germline acquires RNAs or other molecules from the soma, remain unclear.

We are proposing that somatic small RNAs can be directly transported to the germline - through extracellular vesicles. This proposition is based on a recent report showing that dsRNA injected into the body cavity of *C. elegans* can be transported into oocytes through extracellular vesicles derived from intestinal cells. These are the vesicles that usually transport the yolk from the intestine to the embryo, and for this reason, we have developed a sorting strategy to collect large number of vesicles carrying yolk to perform RNA sequencing and proteomics. Our preliminary results, suggest that these vesicles indeed contain endogenous dsRNA molecules and other type of small RNAs, suggesting the existence- of soma-to-germline transfer of small RNAs.

Based on these results, we propose to elucidate the upstream factors and the downstream effectors responsible for this small RNA trafficking, from the intestine to the germline to the next generation. We also propose to study the function of this RNA communication system. Our data show that the gene targeted by these vesicle small RNAs are usually repressed during stress responses. Therefore, we will test whether this is a general mechanism used by the intestine (which is an organ in direct contact with pathogens) to transmit the memory of stress responses across generations.

This project is highly multidisciplinary and will allow the student to acquire diverse skills ranging from molecular biology (generation of transgenic C. elegans strains by CRISPR), biochemistry (immunoprecipitation and purification of protein complexes), developmental biology (phenotypic characterization of progressive sterile worms), imaging (monitoring transcriptional responses to heritable small RNAs in living C. elegans animals by microscopy and FACS sorting).

Tutor/supervisor

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

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 **22**, 235–245.
Cover Article, free online text: https://rdcu.be/b1fm7

Singh M, Cornes E, Li B, Quarato P, Bourdon L, Dingli F, Loew D, Proccacia S, Cecere G. *Translation and codon usage regulate Argonaute slicer activity to trigger small RNA biogenesis.* **bioRxiv** (2020).
doi: https://doi.org/10.1101/2020.09.04.282863

Quarato P, Singh M, Cornes E, Li B, Bourdon L, Didier C, Cecere G. *Argonaute catalytic activity is required for maternal mRNA clearance in embryos.* **bioRxiv** (2020).
doi: https://doi.org/10.1101/2020.02.03.919597

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

Scientific or technical background required for work program

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

Title of the work program 8

Holographic light stimulation of the cochlea to study auditory processing

Description of the work program

Processing of auditory information in the brain is complex because information not only flows from the auditory periphery to the central nervous system but also from the brain to the ear. As a result, efferent neuronal signals can modulate the mechanical properties of the cochlea. Ideally, we would like to know the cochlear output precisely to study its effect on neural representations. However, because cochlear mechanics and neuronal processing are reciprocally coupled through mechanoelectrical feedback, it will require specific tools to uncouple them and to decode the transformation of complex acoustic stimuli by the brain.
The aim of this project is to study how information about sound frequency is propagated from the auditory periphery to the cortex. To understand how sound features are encoded in the brain we need to vary specific parameters of the input and measure how it affects neuronal firing. Recent progress in optogenetics have allowed to activate neuronal circuits precisely. Here we will use these tools to control the cochlear output and realize the first optogenetic activation of cochlear hair cells *in vivo*. Optical methods allow to focalize the beam of a laser onto several cellular targets and rapidly update the temporal pattern of stimulation. The student will design a setup based on holographic light patterning to be able to stimulate simultaneously but independently single hair cells with millisecond precision.

Tutor/supervisor

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

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

7. Barral J, Dierkes K, Lindner B, Jülicher J, and Martin P (2010). Coupling a sensory hair-cell bundle to cyber clones enhances nonlinear amplification. **P.N.A.S.** 107 : 8079-8084

Scientific or technical background required for work program

We want to study how sensory information is encoded in the brain. We are looking for individuals with enthusiasm, curiosity, ambition, and happy to work in a team.
Experience in the following areas: *in vivo* electrophysiology, 2-photon imaging, optogenetics, microscopy, computer science is a plus.

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Title of the work program 9

Impacts of mechanical forces on *Entamoeba histolytica'* invasive processes of the gut

Description of the work program

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

One of our aims is to know whether the intestine mechanical forces (like the peristalsis) could influence *E. histolytica* invasive process (for examples: its adhesion, expression of virulent factors and kinetic of invasion). To answer this question, we propose using **organ-on-a-chip technology** (3D structure) **under stretch** to determine the impact of mechanical forces on *E. histolytica* invasive process. The mathematicians of the lab will compute the tissue stress maps.

- We will analyze and compare *Entamoeba* invasive process over the time on the gut-on-chip with and without stretch, with and without the mucus layer.
- We will use Caco2 cells-on-chip and human colon organoid-on-chip.
- To monitor quantitatively the dynamics of *E. histolytica* intestinal invasion and determine the adhesion sites, we will acquire images with spinning disk and confocal microscopes, and will use fluorescent amoeba (cell tracker) and epithelial biological markers, on live and fixed samples. We will also quantify some cytokines secretion.
- To distinguish what are the critical steps enhancing *E. histolytica* infections we will used deficient amoeba or purified factors known to be involved in the infectious process.

Tutor/supervisor

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

1. Labruyère E, Thibeaux R, Olivo-Marin JC, Guillén N. Crosstalk between *Entamoeba histolytica* and the human intestinal tract during amoebiasis. (2017). Review. Parasitology.

2. A. Boquet-Pujadas, T. Lecomte, M. Manich, R. Thibeaux, E. Labruyère, N. Guillén, J-C Olivo-Marin, and A. C. Dufour. BioFlow: a non-invasive, image-based method to measure speed, pressure and forces inside living cells. (2017). Nature Scientific report

3-Thibeaux R, Avé P, Bernier M, Morcelet M, Frileux P, Guillén N, Labruyère E. The parasite *Entamoeba*

histolytica exploits the activities of human matrix metalloproteinases to invade colonic tissue. (2014). Nat Commun.

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

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

Scientific or technical background required for work program

-
- Cell biology or Microbiology.

Title of the 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 Alzari 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 the early steps of division [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.

Tutor/supervisor

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

1. Sogues, A. et al. Essential dynamic interdependence of FtsZ and SepF for Z-ring and septum formation in Corynebacterium glutamicum. Nat Commun 11, 1–14 (2020).

2. Bellinzoni, M., Wehenkel, A. M., Duran, R. & Alzari, P. M. Novel mechanistic insights into physiological signaling pathways mediated by mycobacterial Ser/Thr protein kinases. Genes & Immunity 1–11 (2019).

3. Akendengue, L. *et al.* Bacterial kinesin light chain (Bklc) links the Btub cytoskeleton to membranes. *Sci. Rep.* 1–10 (2017).

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 11

Synaptic and circuit mechanisms of timing in the brain

Description of the work program

Critical and general to brain function is the necessity to represent changes in sensory stimuli over time, which in turn influence behavior. Time-dependent features such as delays, intervals, and durations of stimuli are encoded by both single neurons (e.g. integrators) and neuronal populations (population clocks) in multiple brain regions. However, a deep understanding of the causal mechanisms generating neural dynamics that encode *time* is lacking.

The cerebellum has long been thought to be a brain region necessary for learning temporal intervals in the subsecond range. Cerebellum's simple neuronal connectivity and cytoarchitecture has inspired elegant models of intrinsic temporal representations and time-dependent associative learning. Its simplicity also makes it a tractable experimental preparation to explore the synaptic and neuronal mechanisms underlying neural circuit dynamics driving behavior. We discovered that synapses conveying sensory information to the cerebellum exhibit diverse amplitudes and short-term plasticity in a sensory-specific manner and can be used to encode the timing of neuronal spikes. **We, therefore, hypothesize that synaptic diversity in the cerebellar cortex is a substrate for a population clock that is used to generate precisely timed behaviors.** In our research we focus particularly on non-motor cerebellum, where multisensory processing is thought to be important for cognitive behaviors and is an area thought to be disrupted in autism and schizophrenia.

The topics of projects in the laboratory include: 1) identification of nanoscale mechanisms of functional synaptic diversity, 2) quantification of the multisensory synaptic diversity in non-motor cerebellum, 3) exploration of the influence of dendritic mechanisms for cerebellar cortical circuit function, 4) monitoring neuronal circuit behavior in awake behaving animals, and 5) establishment of a causal relationship between synaptic and neuronal mechanisms during behavior. Mathematical modeling and big data analysis projects are also available in collaboration with statistical physicists.

Experimental projects in the laboratory use electrophysiology, both in brain slice (patch clamp) and in vivo (Neuropixels). Such experiments are often combined with one or more of the following imaging techniques: super-resolution imaging of macromolecular complexes in presynaptic terminals as well as two-photon imaging of neuronal and synaptic activity using state of the art fluorescence indicators for calcium, neurotransmitter, and membrane voltage both in acute brain slices and *in vivo*. Optogenetic strategies will be used to reversibly alter neuronal activity, and establish causal relationships between mechanisms and behavior. The laboratory is multidisciplinary with in situ and in vivo neurophysiologists, physicists (optics), and theoretical neuroscientists working in a highly collaborative environment. We are in search of highly motivated students interested in exploring **how cellular and molecular diversity of synapses contribute to the neural circuit dynamics controlling behavior**.

Internship projects range from: 1) electrophysiology and imaging of single synapses in brain slices, 2) neural circuit tracing using virus-based fluorescence strategies, 3) modeling synaptic dynamics or nanoscale diffusion-reaction, and 4) analysis "big data sets" acquired in vivo (Neuropixels, behavior, and fluorescence).

Tutor/supervisor

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

Rebola, N., Reva, M., Kirizs, T., Szoboszlay, M., Moneron, G., Nusser, Z. and DiGregorio, D.A. Distinct nanoscale calcium channel and synaptic vesicle topographies contribute to the diversity of synaptic function. ***Neuron***, 104(4): 693-710 (2019). Featured Article and see Preview.

Tran-Van-Minh, A. Rebola, N., Hoehne, A., and DiGregorio, D.A. Two-Photon Neurotransmitter Uncaging for the Study of Dendritic Integration (pgs 33-64). In: Hartveit, Espen. 2019. Multiphoton Microscopy. ***Springer Nature***. 364 pgs. ISBN: 978-1-4939-9701-5. (2019)

Fekete, A., Nakamura Y., Yang, Y-M., Herlitze, S., Mark, M. D., DiGregorio, D. A., and Wang, L-Y. Underpinning heterogeneity in synaptic transmission by presynaptic ensembles of distinct morphological modules. ***Nature Communications,*** 10 (826) (2019).

Marvin, J. S., Scholl, B., Wilson, D.E., Podgorski, K., Kazemipour, A., Mueller, J.A., Schoch-McGovern, S., Wang, S. S-H., Urra Quiroz, F. J., Rebola, N., Bao, B., Little, J. P., Tkachuk, A.N. Hantman, A., Chapman, E.R., Dietrich, D., DiGregorio D.A., Fitzpatrick, D., Looger L.L. Stability, affinity and chromatic variants of the glutamate sensor iGluSnFR ***Nature Methods***, Nov;15(11):936-939 (2018).

Koukouli, F., Rooy, M., Tziotis, D., Sailor K. A., O'Neill H., Levenga, J., Witte, M., Nilges, M., Changeux, J.P., Hoeffer, C., Stitzel, J., Gutkin, B., DiGregorio, D. A., and Maskos, U. Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. ***Nature Medicine***, Mar;23(3):347-354, doi: 10.1038/nm.4274 (2017).

Tran-Van-Minh, A., Abrahamsson, T., Cathala, L. and DiGregorio, D.A. Differential integration of presynaptic activity by dendritic Ca2+ and voltage in cerebellar interneurons. ***Neuron*** 91(4):837-50. doi: 10.1016/j.neuron.2016.07.029 (2016). (See Highlights in same issue)

Chabrol, F.P., Arenz, A., Weichert, M.T., Margrie, T.W. and DiGregorio, D.A. Synaptic diversity enables temporal coding of coincident multisensory inputs in single neurons. ***Nature Neuroscience*** 18(5):718-27. doi: 10.1038/nn.3974 (2015). See also News and Views highlight.

Scientific or technical background required for work program

Good knowledge of synaptic and neuronal physiology using either or both electrophysiology or imaging. Programming and analytical skills are preferable but not required. A genuine interest in how neural circuits process information driving behavior.

Title of the work program 12

Exploring cellular self-organization substrate dimensionality

Description of the work program

The understanding of the processes of self-organization of cells into three-dimensional multicellular structures might provide clues for organ (mal)function therapies and new perspectives for tissue engineering. During cell cycle and in their native environment, cells are affected by membrane tension. Membrane tension is associated to cell-cell and cell matrix interactions and counterbalances that in turn send signals for global and local cytoskeleton organization and regulation. This is one of the main interests of our unit. In the lab, we are using a combination of cell biology and high-resolution light and in-situ cellular cryo-tomography to understand the cytoskeleton reorganization at the nanometer scale. Here, we are offering to interested candidates to use computer-aided design of constrained spatial boundary conditions to induce self-organization in vitro. The ideal candidate holds basic knowledge in physic and cell biology. It will be an advantage if he/she is familiar with light microscopy and computer platforms for graphic design.

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

Marston DJ, Anderson KL, Swift MF, et al. High Rac1 activity is functionally translated into cytosolic structures with unique nanoscale cytoskeletal architecture. Proc Natl Acad Sci U S A. 2019;116(4):1267–1272. doi:10.1073/pnas.1808830116

Engel, L., Gaietta, G., Dow, L. P., Swift, M. F., Pardon, G., Volkmann, N., Weis, W. I., Hanein, D., and Pruitt, B. L. (2019). Extracellular matrix micropatterning technology for whole cell cryogenic electron microscopy studies. J Micromech Microeng 29, 115018 (9pp). doi:10.1101/657072

Scientific or technical background required for work program

Toro-Nahuelpan M, Zagoriy I, Senger F, Blanchoin L, Théry M, Mahamid J. Tailoring cryo-electron microscopy grids by photo-micropatterning for in-cell structural studies. Nat Methods. 2020;17(1):50–54. doi:10.1038/s41592-019-0630-5

Laurent, J., Blin, G., Chatelain, F., Vanneaux, V., Fuchs, A., Larghero, J., and Théry, M. (2017). Convergence of microengineering and cellular self-organization towards functional tissue manufacturing. Nat Biomed Eng 1, 939-956.

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Title of the work program 13

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).
This project will aim at developing single-particle tracking that will integrate machine-learning for finding the optimal associations between moving particles.

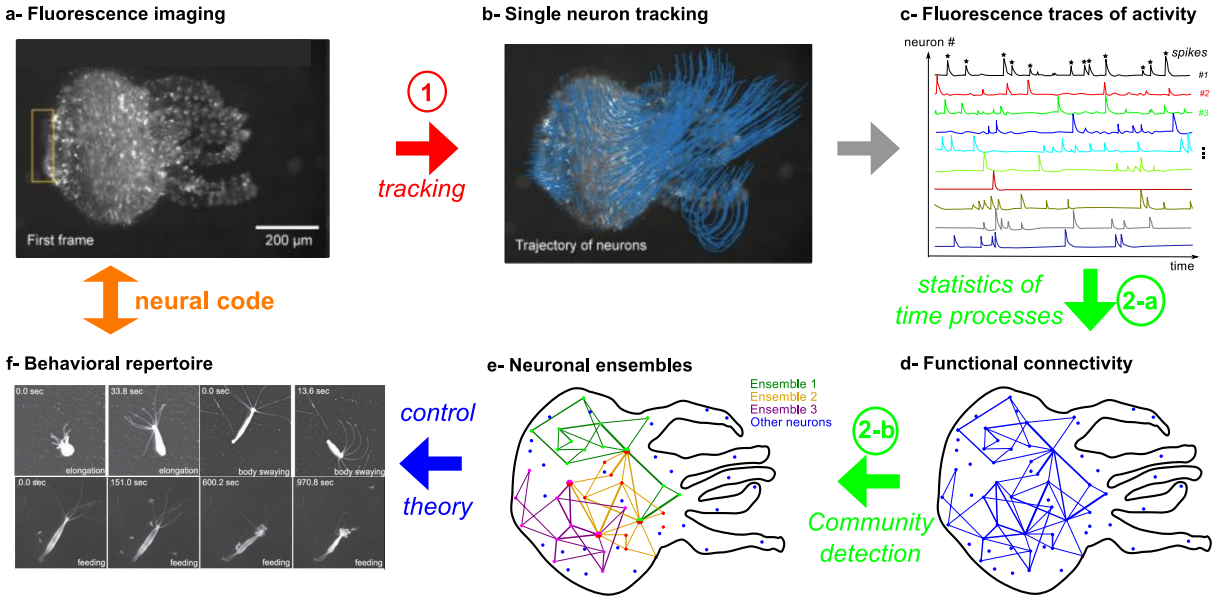

Figure 1: ***Breaking*** **the neural code of Hydra** consists in relating the sequential activity of neurons or ensemble of neurons, observed with fluorescence imaging (**a**) to each specific action of the animal (**f**), as they document significant relations of causality between the time series of 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**). Methodological developments (aims 1&2) are highlighted in red and green.

Tutor/supervisor

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

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

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

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.

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 arificial intelligence is needed. Programming skills (java and/or python) are also required.

Title of the work program 14

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

Description of the work program

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

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

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

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

Objectives

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

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

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

2. Investigate the role of IL-23 in inflammatory disease

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

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2. Langrish, C.L., *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* **201**, 233-240 (2005).
3. Bianchi, E., *et al.* The IL-23/IL-17 pathway in human chronic inflammatory diseases-new insight from genetics and targeted therapies. *Genes Immun* **20**, 415-425 (2019).

4. Coffre, M., *et al.* Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. *Arthritis Rheum* **65**, 1510-1521 (2013).
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8. Venken, K., *et al.* RORgammat inhibition selectively targets IL-17 producing iNKT and gammadelta-T cells enriched in Spondyloarthritis patients. *Nat Commun* **10**, 9 (2019).
9. Baeten, D., *et al.* Secukinumab, an Interleukin-17A Inhibitor, in Ankylosing Spondylitis. *N Engl J Med* **373**, 2534-2548 (2015).
10. Baeten, D., *et al.* Risankizumab, an IL-23 inhibitor, for ankylosing spondylitis: results of a randomised, double-blind, placebo-controlled, proof-of-concept, dose-finding phase 2 study. *Ann Rheum Dis* **77**, 1295-1302 (2018).
11. Aggarwal, S., *et al.* Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J Biol Chem* **278**, 1910-1914 (2003).
12. Yahia-Cherbal, H., *et al.* NFAT primes the human RORC locus for RORgammat expression in CD4(+) T cells. *Nat Commun* **10**, 4698 (2019).
13. Taurog, J.D., *et al.* Ankylosing Spondylitis and Axial Spondyloarthritis. *N Engl J Med* **374**, 2563-2574 (2016).
14. Stoeckius, M., *et al.* Simultaneous epitope and transcriptome measurement in single cells. *Nat Methods* **14**, 865-868 (2017).
15. Cua, D.J., *et al.* Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**, 744-748 (2003).

Tutor/supervisor

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

1. Blijdorp ICJ, Menegatti S, van Mens LJJ, van de Sande MGH, Chen S, Hreggvidsdottir HS, Noordenbos T, Latuhihin TE, Bernink JH, Spits H, Rogge L, Baeten DLP, Yeremenko NG (**2019**) Expansion of Interleukin-22- and Granulocyte-Macrophage Colony-Stimulating Factor-Expressing, but Not Interleukin-17A-Expressing, Group 3 Innate Lymphoid Cells in the Inflamed Joints of Patients with Spondyloarthritis. *Arthritis & Rheumatology* 71: 392-402
2. Menegatti S, Bianchi E, Rogge L (**2019**) Anti-TNF Therapy in Spondyloarthritis and Related Diseases, Impact on the Immune System and Prediction of Treatment Responses. *Frontiers in Immunology* 10: 382.
3. Bianchi E, Rogge L (**2019**) The IL-23/IL-17 pathway in human chronic inflammatory diseases-new insight from genetics and targeted therapies. *Genes & Immunity* 20: 415-425.
4. Yahia-Cherbal H, Rybczynska M, Lovecchio D, Stephen T, Lescale C, Placek K, Larghero J, Rogge L, Bianchi E (**2019**) NFAT primes the human RORC locus for RORgt expression in CD4+ T cells. *Nature Communications*, 10: 4698.
5. Latis E, Michonneau D, Leloup C, Varet H, Peffault de Latour R; CRYOSTEM Consortium, Bianchi E, Socié G, Rogge L (**2020**). Cellular and molecular profiling of T-cell subsets at the onset of human acute GVHD. *Blood Advances* 4: 3927-3942.

Scientific or technical background required for work program

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

Erasmus+

Institut Pasteur 2020-2021

Title of the work program 15

Unraveling the tubulin code

Description of the work program

Microtubules are essential components of the cell cytoskeleton composed of alpha and beta-tubulin heterodimers playing important roles in many cellular processes. Specialisation of tubulin functions is governed by the so-called "tubulin code", where tubulin post-translational modifications provide distinct structural and functional properties to microtubules. Notably, glutamylation corresponding to the addition of one or more glutamates forming a side chain is one of the most abundant modifications, especially represented in cilia and flagella. Trypanosoma brucei is an ideal model to study the tubulin code since their cytoskeleton relies mostly on microtubules and the tubulin code is less complex, facilitating interpretation. Our group has identified several tubulin tyrosine ligase like enzymes (TTLL) potentially involved in glutamylation and the first knockout studies, combined with proteomic analysis revealed unexpected contributions of one particular flagellar TTLL to this process. The aim of this project will be focussed on using tetracycline-inducible expression system for T. brucei to ascertain the role, and glutamylation signature, afforded by this TTLL enzyme. Using well established genetic manipulations of this cell, together with the use of expansion microscopy (ExM), a recently and very powerful developed technique enabling imaging with nanoscale precision by standard fluorescence microscopy, this project will provide key informations about this TTLL enzyme function in T.brucei and importantly will help to decipher the distribution of the polyglutamylation tubulin modifications along individual microtubule doublets of the axoneme and their possible role during intraflagellar transport

Tutor/supervisor

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

1. BERTIAUX, E., MALLET, A., ROTUREAU, B and BASTIN, P. (2020). Intraflagellar transport during assembly of flagella of different length in *Trypanosoma brucei* isolated from tsetse flies. **J Cell Sci.** 2020 Sep 23;133(18):jcs248989. doi: 10.1242/jcs.248989.

2. HUET, D., BLISNICK, T., PERROT, S., and BASTIN, P. (2019). IFT25 is required for the construction of the trypanosome flagellum. **J Cell Sci** *132*. doi: 10.1242/jcs.228296

3. BONNEFOY, S.*, Watson, C.M.*, Kernohan, K.D., LEMOS, M., HUTCHINSON, S., Poulter, J.A., Crinnion, L.A., Berry, I., Simmonds, J., Vasudevan, P., O'Callaghan, C., Hirst, R.A., Rutman, A., Huang, L., Hartley, T., Grynspan, D., Moya, E., Li, C., Carr, I.M., Bonthron, D.T., Leroux, M., Care4Rare Canada, C., Boycott, K.M., BASTIN, P.*, and Sheridan, E.G.* (2018). Biallelic Mutations in LRRC56, Encoding a Protein Associated with Intraflagellar Transport, Cause Mucociliary Clearance and Laterality Defects. **Am J Hum Genet** *103*, 727-739.

4. BERTIAUX, E.*, MORGA, B.*, BLISNICK, T., ROTUREAU, B., and BASTIN, P. (2018). A Grow-and-Lock Model for the Control of Flagellum Length in Trypanosomes. **Curr Biol** *28*, 3802-3814 e3803.
Highighted in "Le Journal de la Recherche"

5. BERTIAUX, E., MALLET, A., FORT, C., BLISNICK, T., BONNEFOY, S., JUNG, J., LEMOS, M., Marco, S., Vaughan, S., Trepout, S., Tinevez, J.Y., and BASTIN, P. (2018). Bidirectional intraflagellar transport is restricted to two sets of microtubule doublets in the trypanosome flagellum. **J Cell Biol** *217*, 4284-4297.
*Highlighted in F1000. Top 5% score of most visible articles on Altmetrics. Comment in: Avasthi, P. **J. Cell Biol** 217:4055-4056.*

6. FORT, C., BONNEFOY, S., Kohl, L., and BASTIN, P. (2016). Intraflagellar transport is required for the maintenance of the trypanosome flagellum composition but not its length. **J Cell Sci** *129*, 3026-3041.

7. SUBOTA, I., JULKOWSKA, D., VINCENSINI, L., REEG, N., BUISSON, J., BLISNICK, T., HUET, D., PERROT, S., SANTI-ROCCA, J., Duchateau, M., Hourdel, V., Rousselle, J.C., Cayet, N., Namane, A., Chamot-Rooke, J., and BASTIN, P. (2014). Proteomic analysis of intact flagella of procyclic Trypanosoma brucei cells identifies novel flagellar proteins with unique sub-localization and dynamics. **Molecular & Cellular Proteomics :** *13*, 1769-1786.

8. ROTUREAU, B., OOI, C.P., HUET, D., PERROT, S., and BASTIN, P. (2014). Forward motility is essential for trypanosome infection in the tsetse fly. **Cell Microbiol** *16*, 425-433.

9. HUET, D., BLISNICK, T., PERROT, S., and BASTIN, P. (2014). The GTPase IFT27 is involved in both anterograde and retrograde intraflagellar transport. **eLife** *3*, e02419.
Highlighted in F1000.

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

Scientific or technical background required for work program

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

Title of the work program 16

Mode of action of Bismuth in the treatment of the infections by the bacterial pathogen *Helicobacter pylori*

Description of the work program

The bacterium *Helicobacter pylori* chronically infects half of the human population. Gastric infection by *H. pylori* causes gastritis, and peptic ulcer disease and *H. pylori* is till now the only bacterium recognised as associated with cancer. Gastric cancer is responsible for 800,000 deaths worldwide and 5,000 in France, annually. However, only 1% of all *H. pylori*-infected individuals show any malignant gastric condition development which indicates a multifactorial process involving host and environmental factors.
The classical treatment against *H. pylori* infection combines two antibiotics and a proton pump inhibitor. However, this therapy has progressively lost efficacy because of an increasing number of antibiotic-resistant strains in patients. *H. pylori* presents a high mutation rate and natural competence that increases the likelihood of acquired resistance to antibiotics. Accordingly, *H. pylori* was included in the recent WHO list of antibiotics (Ab)-resistant "priority pathogens" that pose the greatest threat to human health. Since a few years, a novel medication (Pylera in France) combining antibiotics with a metal, bismuth has proven a higher efficacy in the treatments against *H. pylori* infections. However, the precise mode of action of this metal is still unknown, and the possible impact of bismuth resistant mutants on the Pylera treatment is unknown. We selected several bismuth resistant strains and using next generation sequencing identified putative candidates involved in bismuth resistance.
Our general aim is to establish the molecular basis of the efficacy of Pylera by analysing the activity of bismuth on *H. pylori* laboratory and clinical strains, search for its targets and examine *H. pylori* strains resistant to its toxicity.

For the Erasmus program, the student will be involved in the identifying the roles of genes identified by whole genome seq in bismuth resistance. The interested student will learn to cultivate *H. pylori* in different growth mediums, perform genetic manipulation of *H. pylori* involving gene deletions and complementation and measure the metal transport and resistance properties of these different strains. Various molecular biology techniques will be used in the experimental plan.

The project will be supervised by a Post-Doctoral researcher (Sumith Kumar), who developed this project since two years along with the supervision of Hilde De REUSE, Head of the Unité Pathogenèse de Helicobacter, Department of Microbiology at the Institut Pasteur.

Tutor/supervisor

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

- Fischer F, Robbe-Saule M, Turlin E, Mancuso F, Michel V, Richaud P, Veyrier FJ, De Reuse H, Vinella D, Characterization in Helicobacter pylori of a Nickel Transporter Essential for Colonization That Was Acquired during Evolution by Gastric Helicobacter Species, PLoS Pathog. 2016 Dec;12(12):e1006018.

- D. Vinella, F. Fischer, E. Vorontsov, J. Gallaud, C. Malosse, V. Michel, C. Cavazza, M. Robbe-saule, P. Richaud, J. Chamot-rooke, C. Brochier-armanet, H. De Reuse, Evolution of Helicobacter: Acquisition by Gastric Species of Two Histidine-Rich Proteins Essential for Colonization, PLoS Pathog 11(12): e1005312. doi:10.1371/journal. ppat.1005312.

Scientific or technical background required for work program

Student should be able to communicate well in English. Experimental skills involving culturing of bacteria and cloning is needed for the training.

Title of the work program 17

Understanding the evolution of Yersinia pestis as a highly virulent clone that emerged from the entheropathogen ancestor Yersinia pseudotuberculosis

Description of the work program

Yersinia pestis, the causative agent of plague, has recently diverged from the less virulent enteropathogen *Y. pseudotuberculosis*. Its emergence has been characterized by genetic loss and inactivation of functions associated with enteric disease. The project will consist of studying certain natural mutations present in *Y. pestis* but absent from *Y. pseudotuberculosis*. The impact of the mutations will be evaluated by comparing the capacity of recombinant *Y. pestis* and *Y. pseudotuberculosis* carrying reciprocal mutations to produce either bubonic plague or gastroenteritis. Virulence will be studied in vitro using epithelial cells for bacteria/cell interactions as well as in vivo in mice using both an intradermal injection to mimic flea bite leading to bubonic plague and an oral infectious model to mimic natural gastroenteritis.

Tutor/supervisor

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

1- Bread feeding is a robust and more physiological entheropathogen administration method compared to oral gavage. Derbise et al. infect Immun. 2020. 88(4):810-19

2- Real-Time monitoring of Yersinia pestis promoter activity by bioluminescence imaging
Derbise et al. Methods Mol Biol. 2019. 2010:85-97

3- Inheritance of the lysozyme inhibitor Ivy was an important evolution step by Yersinia pestis to avoid the host innate immune response. Derbies et al. J Infect. Dis. 2013. 207(10):1535-43

Scientific or technical background required for work program

Basic microbiology : bacterial cell culture
Molecular biology : DNA and RNA extraction, PCR

Title of the work program 18

Implication of polymorphisms of the nicotinic receptor α5 subunit gene associated to tobacco smoking in eating disorders

Description of the work program

Eating disorders are multifactorial disorders which represent a major public health problem in most of occidental countries. An association has been identified between eating disorders and tobacco addiction. Such association seems specific to the bulimic dimension. A significant proportion of smokers, in particular teenagers with a negative perception of their body, claim that they smoke to control their weight gain. Several preclinical studies showed that nicotine, the main psychoactive substance of tobacco, alters eating behavior and weight gain. Nicotine directly activates the acetylcholine nicotinic receptors (nAChRs), ionotropic pentamers composed of co-assembling α and α subunits. Over the past years, a set of human genetic studies identified a strong association between a coding polymorphism on the CHRNA5 gene, the rs16969968 (or α5SNP), which encodes the α5 nicotinic subunit, and tobacco addiction. In our team, we have observed that this variant further alters food palatability in transgenic rats constitutively carrying this α5SNP when tested across a self-administration procedure in operant chambers where they have to press a lever to get a small food reward. Human studies showed that this variant is also associated to an increased body mass index (BMI) in non-smokers but, conversely, to a lower BMI in smokers. Interestingly, our preclinical data showed that nicotine exposure could rescue the enhanced food palatability phenotype oberved in rats carrying the α5SNP. These data suggest an implication of CHRNA5 and a strong impact of the α5SNP on eating behavior. We now aim at better characterizing the impact of this variant on eating behavior in our preclinical models, in particular on compulsive eating, and to identify the underlying brain areas and neural mechanisms implicated. For this, we will combine the use of transgenic rodents, behavioral models of bing eating and several approaches for neural activity assessment.

Tutor/supervisor

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

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. 2020 Aug 22:101898

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 Jul 29;177:108234.

Grieder T, Besson M, Maal-Bared G, Pons S, Maskos U, van der Kooy D. Beta2*nAChRs on VTA dopamine and GABA neurons separately mediate nicotine aversion and reward. Proc Natl Acad Sci U S A. 2019 Dec 17;116(51):25968-25973.

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.

A Human Polymorphism in CHRNA5 Is Linked to Relapse to Nicotine Seeking in Transgenic Rats. Forget B, Scholze P, Langa F, Morel C, Pons S, Mondoloni S, Besson M, Durand-de Cuttoli R, Hay A, Tricoire L, Lambolez B, Mourot A, Faure P, Maskos U. Curr Biol. 2018 Oct 22;28(20):3244-3253.e7.

Scientific or technical background required for work program

A strong backgroung in neuroscience is required, preferentially in the neuronal correlates of behavior and cognition and in preclinical models of psychiatic disorders. Previous experience in handling rodents would be welcome.

Title of the work program 19

Patterning precision in the fly eye

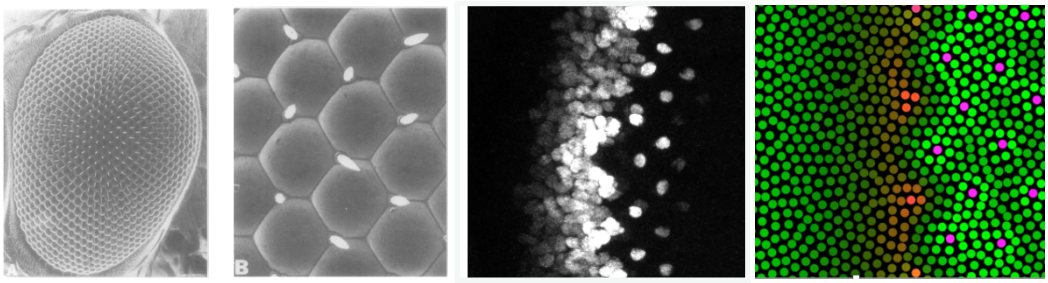
Description of the work program

Our lab is addressing curiosity-driven questions in the field of developmental biology: How do cell acquire different fates during development and produce stereotyped patterns of cell fates? In this context, how does self-organization, e.g. Turing-like process, combine with positional cues instructing cells about their position in the tissue? To decipher the inner logic of fate decisions in time and space, our laboratory is using fruit flies as it provides outstanding tools to examine in a rapid and with unsurpassed temporal and spatial resolutions the effects of controlled perturbations.

We are currently investigating how patterns of cell fates emerge during the development of sensory organs. We have recently shown that a self-organized process mediated by Delta-Notch signaling operates at the tissue scale to produce a stereotyped pattern of bristle in the adult fly (Corson et al., 2017). A similar self-organized process can produce a regular pattern of founder photoreceptor cells, R8, in the eye. Using live imaging, we have discovered that a key and conserved transcription factor involved in generating this pattern is expressed in a pulsatile manner. We hypothesize that these pulses may contribute to the precision of the R8 pattern.

In this project, you will measure through live imaging the precision of the R8 pattern, examine how patterning irregularities introduced through genetic perturbations can be corrected and test the role of transcription factor pulses in achieving precision.

Through this project, you will learn about fly genetics, live imaging, transcription factor dynamics, cell-cell interaction via the Notch receptor and you will discover how multicellular organisms are built through highly precise patterning mechanism.



Tutor/supervisor

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

• Corson, Couturier, Rouault, Mazouni, **Schweisguth**. 2017. Self-organized Notch dynamics generate stereotyped sensory organ patterns in *Drosophila*. **Science** 356, 501, eaai7407
• Trylinski, Mazouni, **Schweisguth**. 2017. Intra-lineage fate decisions involve activation of Notch receptors basal to the midbody in *Drosophila* sensory organ precursor cells. **Current Biology**, 27, 2239-47
• Perez-Mockus, Mazouni, Roca, Corradi, Conte, **Schweisguth**. 2017. Spatial regulation of contractility by Neuralized and Bearded during furrow invagination in *Drosophila*. **Nature Communications**, 8, 1594
• Perez-Mockus, Roca, Mazouni, **Schweisguth**. 2017. Neuralized regulates Crumbs endocytosis and epithelium morphogenesis via specific Stardust isoforms. **The Journal of Cell Biology**, 205, 1405-20
• Henrique, **Schweisguth**. 2019. Mechanism of Notch signaling: a simple logic deployed in time and space. **Development**, 146, doi: 10.1242/dev.172148
• Couturier, Mazouni, Corson, **Schweisguth** . 2019. Regulation of specific *Enhancer of split-HLH* genes by proneural factors shapes Notch output dynamics during bristle patterning in *Drosophila*. **Nature Communications**, 10:3486
• **Schweisguth**, Corson 2019. Self-organization in pattern formation. **Developmental Cell** 49, 556-573

Scientific or technical background required for work program

Training in cell and developmental biology with an interest in microscopy and image analysis
or
Training in physics with an interest in experimental biology and image analysis

Erasmus+

Institut Pasteur 2020-2021

Title of the work program 20

Contribution of sperm ageing in epigenetic signature of *Anopheles* susceptibility to *Plasmodium* malaria parasite

Description of the work program

Malaria is still a public health concern in several tropical developing countries. This disease is caused by *Plasmodium* parasites which are transmitted by *Anopheles* mosquitoes. We have discovered that maternal effects control *Anopheles* susceptibility to the human parasite, *Plasmodium falciparum* with progeny from older females being more susceptible to the parasite and surviving longer. As female mosquitoes mate only once in their life, progeny from old females are also the product of old sperm. In the proposed project, we want to address the contribution of ageing sperm in the susceptibility of progeny to Plasmodium parasite and progeny survival.
The project will use two different approaches : 1. Looking at survival rates from progeny of females of the same age but mated at different time of their life . 2. Investigating epigenetic marks in sperm DNA collected from “young” and “old” mosquito sperm collected from females at different time after copulation, as epigenetic signatures tend to be the hallmark of ageing processes.
The student, with the help of a qualified technician, will be producing different progeny from a same batch of females, but mated at different time of their life and survey their survival rates.
Secondly to address the potential contribution of epigenetics in ageing sperm, the student will purify sperm stored in the spermathecae of different batches of females of the same age but early mated versus late mated. DNA will be extracted from these different batches of sperm and process for epigenetic marks as histone modification using a CHIP seq technology.

Tutor/supervisor

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

1. Fine Pathogen Discrimination within the APL1 Gene Family Protects Anopheles gambiae against Human and Rodent Malaria Species.
2. Mitri, Christian; Jacques, Jean-Claude; Thiery, Isabelle; et al. **Source:** Plos Pathogens **Volume:** 5 **Issue:** 9 **Published:** SEP 2009Mosquito-based transmission blocking vaccines for interrupting Plasmodium development Lavazec, Catherine; Bourgouin, Catherine. Microbes and Infection **Volume:** 10 **Issue:** 8 **Pages:** 845-849 **Published:** JUL 2008
3. Pondeville E, Puchot N, Lang M, Cherrier F, Schaffner F, Dauphin-Villemant C, Bischoff E, **Bourgouin C**. Evolution of sexually-transferred steroids and mating-induced phenotypes in Anopheles mosquitoes. Sci Rep. 2019 Mar 15;9(1):4669. doi: 10.1038/s41598-019-41094-4.PMID: 30874601
4. Goupeyou-Youmsi, J., Rakotondranaivo, T., Puchot, N., Peterson I., Girod R., Vigan-Womas I., Ndiath, M.O., **Bourgouin C.** (2019). "Differential contribution of *Anopheles coustani* and *Anopheles*

arabiensis to the transmission of *Plasmodium falciparum* and *Plasmodium vivax* in two neighboring villages of Madagascar." bioRxiv: 78743, under revision Parasite & Vectors

5. Emilie Pondeville, Nicolas Puchot, JeanPhilippe Parvy, Guillaume Carrissimo, Mickael Poidevin, Ro bert M. Waterhouse, Eric Marois, **Catherine Bourgouin (**2020) Hemocyte-targeted gene expression in the female malaria mosquito using the *hemolectin* promoter from *Drosophila***.** Insect Biochemistry and Molecular Biology, In press, journal pre-proof Available online 24 February 2020Article 103339
6. Christian Mitri, Isabelle Thiery, Marie-Thérèse Lecoq, Catherine Thouvenot, Solange Touron, Annie Landier, Emmanuel Bischoff, **Catherine Bourgouin.** (2020). *Anopheles gambiae* maternal age and parous state control offspring susceptibility to *Plasmodium falciparum*. BioRxiv, doi: https://doi.org/10.1101/2020.01.27.922070

Scientific or technical background required for work program

Malaria is still a public health concern in several tropical developing countries. This disease is caused by Plasmodium parasites which are transmitted by *Anopheles* mosquitoes. We have discovered that maternal effects control *Anopheles* susceptibility to the human parasite, *Plasmodium falciparum* with progeny from older females being more susceptible to the parasite and surviving longer. As female mosquitoes mate only once in their life, progeny from old females are also the product of old sperm. In the proposed project, we want to address the contribution of ageing sperm in the susceptibility of progeny to Plasmodium parasite and progeny survival.
The project will use two different approaches : 1. Looking at survival rates from progeny of females of the same age but mated at different time of their life . 2. Investigating epigenetic marks in sperm DNA from “young” and “old” mosquito sperm collected from females at different time after copulation, s epigenetic signatures tend to be the hallmark of ageing processes.
The student, with the help of a qualified technician, will be producing different progeny from a same batch of females, but mated at different time of their life and survey their survival rates.
Secondly to address the potential contribution of epigenetics in ageing sperm, the student will purify sperm stored in the spermathecae of different batches of females of the same age but early mated versus late mated. DNA will be extracted from these different batches of sperm and process for epigenetic marks as histone modification using a CHIP seq approaches

No specific technical skills for this project except motivation to work on mosquitoes and acceptance of delicate experiments. Training will include mosquito production, dissection, DNA purification and depending on the skill and time CHIP-Seq experiments. FACS sorting for purifying sperm will be envisaged.
The student will be hosted in C. Bourgouin group, member of the Functional Genetics of Infectious Diseases Unit directed by Anavaj Sakuntabhai. The Unit belongs to the Global Health department that will offer broad interaction with many PhD students and postdocs, as well as with junior and senior scientists of the department

Title of the work program 21

Exploring clinical metagenomic data from mothers and infants using machine learning with python to understand infection and antibiotic resistance

Description of the work program

The internship project will be performed in the context of the Innovative Strategies for Perinatal Infection Risk-Reduction (InSPIRe) project. InSPIRe is an ambitious collaboration including teams from Paris hospitals, research institutes and the private sector. The primary goal of InSPIRe is to identify biomarkers of perinatal infections and antibiotic resistance to develop a medical device that can detect these markers in < 15 minutes at the time of delivery. Dr. Kennedy's group, in the Computational Biology Department at the Institut Pasteur, is responsible for the sequencing and bioinformatics analysis of the clinical samples for this study. 2000 samples will be analyzed by shotgun sequencing. Our team is constructing and annotating a comprehensive vaginal microbiome reference gene catalog with data form clinical samples. We use both standard statistical methods as well as machine learning to identify risks of infection and predict at-risk pregnancy outcomes such as pre-term birth or premature rupture of the amniotic membrane.
The student's project will include both the development of analysis tools as well as the use of these pipelines for data analysis for the InSPIRe project. Our team has developed an open source Python library, moonstone (https://github.com/motleystate/moonstone), dedicated to the analysis of metagenomics gene counts. This Python library aims to contain a comprehensive set of 'bricks' necessary to perform an analysis from statistical tests to visualization. and the student will contribute to its construction. This work, including gaining a better understanding of good programming and development skills, will be tightly related to biological questions.
The exposure to Python development in the context of a clinical metagenomics study will provide a valuable career experience and a rich opportunity for interactions with the host lab the InSPIRe project consortium and other teams at Institut Pasteur.

Tutor/supervisor

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

1. Volant, S. *et al.* SHAMAN: a user-friendly website for metatagenomic analysis from raw reads to statistical analysis. *BMC Bioinformatics* **21**, 345 (2020).
2. Ruppe, E. *et al.* Prediction of the intestinal resistome by a three-dimensional structure-based method. *Nature microbiology* **4**, 112-+ (2019).
3. Lanza, V. F. *et al.* In-depth resistome analysis by targeted metagenomics. *Microbiome* **6**, 11 (2018).

4. de Gunzburg, J. *et al.* Protection of the Human Gut Microbiome From Antibiotics. *J Infect Dis* **217**, 628–636 (2018).
5. Quereda, J. J. *et al.* Bacteriocin from epidemic Listeria strains alters the host intestinal microbiota to favor infection. *Proc Natl Acad Sci U S A* **113**, 5706–11 (2016).
6. Chatelier, E. L. *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541–546 (2013).

Scientific or technical background required for work program

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

Erasmus+

Institut Pasteur 2020-2021

Title of the work program 22

How does transglutaminase 2 regulate gene expression in cancer cells?

Description of the work program

Tissue transglutaminase (TG2) is an ubiquitously expressed enzyme. It is highly expressed in most cancers and there is some indication that its transamidase activity might be rather tumor suppressive. However, the global consequence of TG2 activation in cancer cells is largely unclear. The transamidase activity results in post-translational modifications (PTM) of specific protein targets through a calcium-dependent reaction. It was recently showed that histone 3 (H3) is one substrate of TG2, and that histone 3 serotonylation by TG2 affects development. The host lab independently showed that the transamidase activity of TG2 was required for the regulation of expression of at least two important metabolic genes. The student will test the hypothesis that this regulation of the expression of these genes by TG2 involves H3 serotonylation. Furthermore, we will investigate the global consequence of TG2 activation on gene expression, and the molecular basis of this pattern.

Tutor/supervisor

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

1. Hamaoui, D, Cossé, M.M., Mohan, J. Lystad, A.H., Wollert, T. and Subtil, A. The *Chlamydia* effector CT622/TaiP targets a non-autophagy related function of ATG16L1 (2020) **PNAS** *in press* doi:10.1073/pnas.2005389117
2. Maffei, B., Laverriere, M., Wu, Y., Triboulet, S., Perrinet, S., Duchateau, M., Matondo, M., Hollis, R. L., Gourley, C., Rupp, J., Keillor, J. W., and Subtil A Infection-driven activation of transglutaminase 2 boosts glucose uptake and hexosamine biosynthesis in epithelial cells(2020). **EMBO J.** 39, e102166 doi 10.15252/embj.2019102166
3. Cossé M.M., Barta ML, Fiser DJ, Oesterlin LK, Niragire B, Perrinet S, Millot GA, Hefty PS, Subtil A The loss of expression of a single type 3 effector (CT622) strongly reduces *Chlamydia trachomatis* infectivity and growth (2018) **Front Cell Infect Microbiol**. 8:145 doi 10.3389/fcimb.2018.00145
4. Vromman F, Perrinet S, Gehre L, Subtil A. The DUF582 Proteins of *Chlamydia trachomatis* Bind to Components of the ESCRT Machinery, Which Is Dispensable for Bacterial Growth In vitro. (2016) **Front Cell Infect Microbiol**. Oct 7;6:123..
5. Gehre L, Gorgette O, Perrinet S, Prevost MC, Ducatez M, Giebel AM, Nelson DE, Ball SG, Subtil A Sequestration of host metabolism by an intracellular pathogen. (2016) **Elife**. Mar 16;5:e12552. doi: 10.7554/eLife.12552.

Scientific or technical background required for work program

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

Title of the work program 23

Biogenesis of a parasitic vacuole: role for the ELRO biosynthesis machinery

Description of the work program

Endo-lysosome-related organelles (ELROs) are specialized, functionally and morphologically diverse secretory compartments derived from the endo-lysosomal system to fulfil cell-dependent specific needs. Best studied examples are pigment cell melanosomes, platelet dense and alpha granules, or cytotoxic T cell (CTL) lytic granules. Defects in proteins implicated in ELRO biogenesis result in a wide spectrum of pathologies, which were key to unravel the precise molecular functions of these proteins. Importantly, most of these molecular players are ubiquitously expressed, and co-opted to produce specific ELROs in specialized cells, such as melanocytes, platelets of cytotoxic T lymphocytes.

We have gathered preliminary evidence that some key players in ELROs biogenesis are also exploited by an intracellular pathogen, *Chlamydia trachomatis*, presumably to build its specific vacuolar niche. *C. trachomatis* is a human adapted pathogen that is the first cause of sexually transmitted infection of bacterial origin. The bacteria multiply in a vacuole that intersects host trafficking pathways, in particular originating from the Golgi apparatus. Most of the proteins present on the vacuole membrane, called Inc proteins, are of bacterial origin and are translocated in this membrane by a dedicated secretion mechanism. Because the vacuole membrane is largely devoid of host proteins, while the host contributes most of its lipids, mechanisms to retrieve material from this compartment must exist, that are completely unknown. We have observed that the morphology of the vacuole is modified in cells depleted for several key players of melanosome biogenesis, indicating that the corresponding machineries have been co-opted by the bacteria to retrieve superfluous host proteins and lipids from the vacuole.

The trainee will test this hypothesis by looking at the consequence of depleting key players in melanosome biogenesis (e.g. BLOC-I, KIF13A) on the morphology of the vacuole, and on bacterial proliferation. Sequestration of these molecules on the vacuole periphery will be investigated by microscopy techniques. For positive hits, potential bacterial partners inserted in the vacuole membrane will be searched among Inc proteins through proteomic approaches. Other known machineries involved in protein sorting from intracellular compartments will also be investigated, as several mechanisms may take part in the sorting of host protein at the inclusion membrane.

Tutor/supervisor

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Note: this project will be co-supervised by C. Delevoye at Institut Curie (Paris). CD is a specialist in melanosome biogenesis.	

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

1. Hamaoui, D, Cossé, M.M., Mohan, J. Lystad, A.H., Wollert, T. and Subtil, A. The *Chlamydia* effector CT622/TaiP targets a non-autophagy related function of ATG16L1 (2020) **PNAS** *in press* doi:10.1073/pnas.2005389117
2. Maffei, B., Laverriere, M., Wu, Y., Triboulet, S., Perrinet, S., Duchateau, M., Matondo, M., Hollis, R. L., Gourley, C., Rupp, J., Keillor, J. W., and Subtil A Infection-driven activation of transglutaminase 2 boosts glucose uptake and hexosamine biosynthesis in epithelial cells(2020). **EMBO J.** 39, e102166 doi 10.15252/embj.2019102166
3. Ripoll, L., Heiligenstein, X., Hurbain, I., Domingues, L., Figon, F., Petersen, K. J., Dennis, M. K., Houdusse, A., Marks, M. S.,Raposo, G., and Delevoye, C. Myosin VI and branched actin filaments mediate membrane constriction and fission of melanosomal tubule carriers. **J. Cell Biol.** (2018) 217, 2709-2726
4. Delevoye, C., Heiligenstein, X., Ripoll, L., Gilles-Marsens, F., Dennis, M. K., Linares, R. A., Derman, L., Gokhale, A., Morel, E., Faundez, V., Marks, M. S., and Raposo, G. BLOC-1 Brings Together the Actin and Microtubule Cytoskeletons to Generate Recycling Endosomes. (2016) **Curr. Biol.** 26, 1-13
5. Gehre L, Gorgette O, Perrinet S, Prevost MC, Ducatez M, Giebel AM, Nelson DE, Ball SG, Subtil A Sequestration of host metabolism by an intracellular pathogen. (2016) **Elife**. Mar 16;5:e12552. doi: 10.7554/eLife.12552.

Scientific or technical background required for work program

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

Title of the work program 24

Mechanisms of neuroinvasion of SARS-CoV-2

Description of the work program

Introduction/Background: The entry of SARS-CoV-2 into human host cells is mediated mainly by a cellular receptor angiotensin-converting enzyme 2 (ACE2). The ACE2 receptor protein is mainly expressed on the apical surface of lung alveolar epithelial cells and on enterocytes of the small intestine. However, the presence of ACE2 solely is not sufficient to make host cells susceptible to infection. ACE2-expressing endothelial cells and human intestinal cell lines failed to be infected by SARS-CoV, while some cells without a detectable expression level of ACE2, such as hepatocytes could also be infected by SARS-CoV. Likewise, the infection of SARS-CoV or MERS-CoV was also reported in the central nervous system (CNS), where the expression level of ACE2 is very low under normal conditions (*Yan-Chao Li, Wan-Zhu Bai,Tsutomu Hashikawa, 2020*). The infection of SARS-CoV-2 has been reported in the brains from both patients and experimental animals, where the brainstem was heavily infected. Furthermore, some coronaviruses have been demonstrated able to spread via a synapse-connected route to the medullary cardiorespiratory center from the mechanoreceptors and chemoreceptors in the lung and lower respiratory airways. ***This viral spreading is reminiscent of the spreading of toxic amyloid proteins in neurodegenerative disorders (NDs)*** whereby, for example in the case of Parkinsons disease, alpha-synunclein aggregates move progressively from peripheral sites of infection to the brainstem and up to the cortex explaining the progression of the disease (*Victoria and Zurzolo, JCB 2017*; *Hawkes CH, Del Tredici K, Braak H., 2009*). We have previously shown ***that spreading of amyloid aggregates between cells of the CNS and from peripheral lymphoid system cells to neurons occurs mainly through Tunneling Nanotubes (TNTs) a novel way of direct communication between distant cells*** (*Gousset et al, NCB 2009, Abounit et al, EMBOJ 2016, Vargas et al, EMBOJ 2019*). *Viruses of many different families, including retroviruses, and enveloped viruses like herpesviruses, orthomyxoviruses and several others have been reported to trigger the formation of TNTs or TNT-like structures in infected cells and to use these structures to efficiently spread to uninfected cells.* Because TNT-transferred virions do not need to be exposed outside of the host cell, ***we hypothesize that TNT-mediated transmission is a mechanism for viruses to escape neutralizing antibody activity and immune surveillance, as well as to infect less permissive cells lacking the membrane receptor for virus entry, thus allowing the spreading of tropism and pathogenicity of the viral strain.*** In support of this hypothesis we have set up up co-cultures between cells permissive to infection (VERO E6 and A549-ACE2, as infected donors) and neuronal cells non-permissive to infection (CAD, SHSY5Y, as non-infected acceptors). Recent data from our lab show that SARS-CoV-2 could spread from the infected cells to the non-permissive human neuronal cell line (SHSY5Y). We also found that infection increases the number of TNTs formed between cells in single culture and in cocultures. Furthermore by CryoEM we could see the virus on TNTs (*Pepe et al., in preparation*).

Ìn the proposed project the student will addrress the following questions:

Is SARS-CoV-2 able to replicate in neuronal cells ? To this purpose he/she will apply: 1. classical confocal fluorecence microscopy and superesolution to identy the different viral proteins in coculture after the virus has been neutralized and perform qauntitative image analysis. 2: FISH to identify (again after fixation in co-culture) the positive and negative strand of RNA (being a +strand virus, the presence of -strand would indicate replication of SARS-CoV-2).

Is SARS-CoV-2 able to spread to human neuronal precursors and/or mouse primary neurons? To this purpose the student will help setting co-culture conditions with more physiologically relevant neuronal models (either prepare primary neurons and astrocytes from mouse brains or differenciating Human IPs cells in human neuronal precursors).

Through which cells SARS-CoV-2 reaches the brain? Once demonstrated that neuronal cells can be infected by TNT mediated transfer from other cells, the question arises as to which peripheral cells will allow this transfer to the brain.To this purpose he/she will help setting coculture conditions between primary cells infected cells that we postulate to have a role in the spreding of the viurus to the brain *in vivo* (eg, bronchial epithelial, endothelial, and cells of the immune system, specifically dendritic cells and microglia) and acceptor neuronal cells, and analyse virus spreading.
How SARS-CoV-2 moves in TNTs? Based on our recent ability to image TNT by cryo EM and tomography and FIB SEM (*Sartori et al Nature Communications 2019*), the student will learn how to use these advanced techniqued and the new Titan microscope at the nonoimaging facility at the IP (https://research.pasteur.fr/en/team/nanoimaging/) in order to characterize the structure of the virus and investigate its presence in TNTs. The following techniques will be applied in this project:

- Cell culture (different cell lines, primary neurons, huIPS)
- Infection protocols (the student will not be asked to work with the infectious virus but only with the inactivated one)
- Western blot
- Immunofluorescence
- Confocal microscopy
- Spinning disk microscopy
- Super resolution microscopy
- Cryo EM and tomography (TITAN)
- FIB-SEM
- RNA-FISH
- Quantitative image analysis softwares and tomograms reconstructions (Icy, Imaris, eTomo, IMOD)

Experiments will be designed directly with supervisor and will be increasingly difficult, according to the aims above, depending on the practical and theoretical ability of the student. The student will participate to weekly lab meetings where he/she will present own results and discuss with all lab members. Possibility to write a paper will be given depending of the amounts and quality of the data produced.

Tutor/supervisor

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

1. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, Olivo-Marin JC, Männel D, Zurzolo C. “Prions hijack tunnelling nanotubes for intercellular spread.” Nat Cell Biol. 2009 Mar;11(3):328-36. https://doi.org/10.1038/ncb1841

2. Abounit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R, Zurzolo C "Tunneling nanotubes spread fibrillar α-synuclein by intercellulartrafficking of lysosomes". EMBO J. 2016 Oct 4;35(19):2120-2138. https://doi.org/10.15252/embj.201593411
3. Vargas JY, Loria F, Wu YJ, Córdova G, Nonaka T, Bellow S, Syan S, Hasegawa M, van Woerden GM, Trollet C, Zurzolo C. "The Wnt/Ca2+ pathway is involved in interneuronal communication mediated by tunneling nanotubes". https://doi.org/10.15252/embj.2018101230
4. Sartori-Rupp, A, Cordero Cervantes D, Pepe A, Gousset K, Delage E, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, and Zurzolo C. "Correlative Cryo-Electron Microscopy Reveals the Structure of TNTs in Neuronal Cells." *Nature Communications* 10, no. 1 (December 2019): 342. https://doi.org/10.1038/s41467-018-08178-7

Selected Reviews'

1. Abounit S, Delage E, and Zurzolo C. "Identification and Characterization of Tunneling Nanotubes for Intercellular Trafficking." *Current Protocols in Cell Biology* 67, no. 1 (June 2015). https://doi.org/10.1002/0471143030.cb1210s67.

2. Marzo L, Gousset K and Zurzolo C. "Multifaceted roles of tunneling nanotubes in intercellular communication". https://doi.org/10.3389/fphys.2012.00072

3. Korenkova O, Pepe A, and Zurzolo C. "Fine Intercellular Connections in Development: TNTs, Cytonemes, or Intercellular Bridges?" *Cell Stress* 4, no. 2 (February 10, 2020): 30–43. https://doi.org/10.15698/cst2020.02.212.

4. Ariazi ,…. and Zurzolo C. "Tunneling Nanotubes and Gap Junctions–Their Role in Long-Range Intercellular Communication during Development, Health, and Disease Conditions" https://doi.org/10.3389/fnmol.2017.00333

Scientific or technical background required for work program

We are looking for an excellent curious, energetic and highly motivated candidate, with strong work ethics, interest in cell biology, virology and/or neuroscience and ready to learn and apply forefront imaging techniques to address biological problems. Team-work ability and enthusiasm are necessary, previous experience in imaging and cell culture is welcome, but not absolutely required. Good communication skills are important.

Erasmus+

Institut Pasteur 2020-2021

Title of the work program 25

A novel host target for development of anti-malaria drugs

Description of the work program

Malaria parasites invade and multiply within red blood cells (RBCs) during the blood stage of the parasite life cycle. As the parasites grow and multiply in RBCs, they ingest haemoglobin (Hb), which is digested by proteases to release amino acids and free heme. This process imposes significant oxidative stress on the parasite and host RBCs, which leads to oxidation of lipids and membrane damage. We have preliminary evidence that a host phospholipase A2, peroxiredoxin 6 (PRDX6) is imported by the parasite along with Hb and may play a role in lipid repair during parasite growth. In this project, the student will study transport of PRDX6 from the RBC cytosol to the food vacuole using fluorescence and electron microscopy. The functional role of PRDX6 in membrane repair will also be studied using biochemical and lipidomic approaches. Inhibitors targeting PRDX6 will be tested in biological assays to test inhibition of parasite growth *in vitro* as well as *in vivo* in animal models. These studies have the potential to lead to development of novel anti-malaria drugs that target a host enzyme, which precludes the development of drug resistance.

Tutor/supervisor

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

1. More KR, Kaur I, Giai Gianetto Q, Invergo BM, Chaze T, Jain R, Huon C, Gutenbrunner P, Weisser H, Matondo M, Choudhary JS, Langsley G, Singh S, **Chitnis CE.** 2020. Phosphorylation-dependent assembly of a 14-3-3 mediated signaling complex during red blood cell invasion by *Plasmodium falciparum* merozoites. **mBio.** 11(4):e01287-20. doi: 10.1128/mBio.01287-20.
2. Siddiqui MA, Singh S, Malhotra P, **Chitnis CE**. 2020. Protein S-palmitoylation is responsive to external signals and plays a regulatory role in microneme secretion in *Plasmodium falciparum* merozoites. **ACS Infect Dis**. 6(3):379-392.
3. Singh P, Alaganan A, More KR, Lorthiois A, Thiberge S, Gorgette O, Guillotte Blisnick M, Guglielmini J, Aguilera SS, Touqui L, Singh S, **Chitnis CE**. 2019. Role of a patatin-like phospholipase in *Plasmodium falciparum* gametogenesis and malaria transmission. **Proc. Natl. Acad. Sci. USA.** 116(35):17498-17508.
4. Iyer GR, Singh S, Kaur I, Agarwal S, Siddiqui MA, Bansal A, Kumar G, Saini E, Paul G, Mohmmed A, **Chitnis CE**, Malhotra P. 2018. Calcium-dependent phosphorylation of *Plasmodium falciparum* serine repeat antigen 5 triggers merozoite egress. **J Biol Chem.** 293(25):9736-9746.
5. Dawn A, Singh S, More KR, Siddiqui FA, Pachikara N, Ramdani G, Langsley G, **Chitnis CE. 2014**. The central role of cAMP in regulating *Plasmodium falciparum* merozoite invasion of human erythrocytes. **PLoS Pathog**. 10(12):e1004520.

Scientific or technical background required for work program

Basic knowledge of cell biology, microbiology and protein biochemistry together with a passion for exploring new ideas especially through experiments in the laboratory.

Title of the work program 26

Mapping Tunneling Nanotubes in the Developing Brain using *Connectomics*

Description of the work program

The way in which cells interact with each other is a topic that has fascinated biologists for many centuries. Our group is interested in a form of cell-to-cell communication mechanism that directly connects cells. This connection is known as a Tunneling Nanotube, or TNT.

TNTs are direct anatomical connections between cells found in numerous and distinct types of cells. Unlike other cellular membranous structures (e.g. filopodia), TNTs connect the cytoplasm of distant cells. TNTs vary in diameter and can extend up to over 100 µm in length. These dynamic structures transfer various cellular cargoes such as cytoplasmic molecules, vesicles, and organelles. In addition, TNTs can be "hijacked" by various pathogens such as bacteria, viruses, and prions, in order to spread to neighboring cells. This remarkable functional ability TNTs have to transfer cargo make them relevant for understanding essential physiological and pathological processes.

Our group spent the last decade setting up the tools to study TNTs *in vitro* and demonstrated that they play an important role in the intercellular transfer of amyloidogenic proteins involved in neurodegenerative diseases. By employing state-of-the-art electron microscopy strategies, we also discovered that TNTs in neuronal cells are comprised of a bundle of open-ended individual TNTs (https://www.youtube.com/watch?v=kKcwm1AHZ9g&t=133s). In spite of the wealth of knowledge we have acquired about TNTs in culture however, the field still lacks evidence showing that these structures exist *in vivo* (i.e. in animal tissue).

In order to address this question, this project proposes the identification and characterization of TNT-like structures *in vivo* using an interdisciplinary set of approaches in three specific aims.
Aim 1: Connectomics-based screening of TNTs in the developing brain. By partnering with the Lichtman lab, a group at Harvard University interested in unraveling the brain connectome by using Serial Sectioning Scanning Electron Microscopy, we have generated large 3D image volumes made up of thousands of brain slices. Through manual coloring of cells in the volume via a process called segmentation, we have been able to identify TNT-like structures connecting dozens of cells in the developing mouse brain. 3D Studio Max, (a motion graphic software), has enabled us to observe these structures in 3D and produce movie renditions from the inside of the tube. ***In this aim, the student will learn how to analyze 3D electron microscope image volumes through segmentation and 3D-image visualization to further screen our image volumes for TNT-like structures.***
Aim 2: Structural characterization of TNTs through computational approaches. Leveraging the latest advances in *skeletonization*, a computational process adapted by the Seung lab (Princeton University) to trace the paths of connectomes (https://github.com/seung-lab/kimimaro), we developed a tool for visualization- and morphological analysis of TNT-like structures in connectomes. Using this tool, the student will characterize the TNTs identified in Aim 1 by obtaining their architectural features (e.g. surface area, volume, length, and curvature). ***These local morphologies will allow the student to catalog- and differentiate TNT-like structures from other membranous processes, such as filopodia.***
Aim 3: Functional assessment of TNTs through dye-coupling. To corroborate and support the results obtained in Aim 1 and 2, this aim will test whether neuronal TNT-like structures in the developing mouse brain enable the intercellular passage of dyes. To this end, ***the student will participate in patch-clamping- and dye microinjection experiments of cells of living, organotypic brain tissue slices.***

Moreover, the student will further study the connections identified through immunohistochemical approaches, confocal microscopy, and image analysis.

Taken together, the findings obtained by these experiments have the potential to yield the first-ever identification of TNTs in brain tissue, challenging the historical preconception that neuronal cells only communicate through synapses, and shifting a paradigm in the field of neurobiology that hasn't been questioned since neuroscientist Ramón y Cajal first introduced the neuron doctrine.

Tutor/supervisor

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

Essential:
Sartori-Rupp A., Cordero Cervantes D, Pepe A, Delage E, Gousset K, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, Zurzolo C. **Correlative cryo-electron microscopy reveals the structure of TNTs in neuronal cells**. *Nature Communications*. 2019 Jan 21;10(1):342. doi: 10.1038/s41467-018-08178-7.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/30664666

Abounit S, Zurzolo C, **Wiring through tunneling nanotubes–from electrical signals to organelle transfer**, *J. Cell. Sci*. 2012 Mar;125(Pt 5):1089-98.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/22399801

Victoria GS, Zurzolo C, **The spread of prion-like proteins by lysosomes and tunneling nanotubes: Implications for neurodegenerative diseases**. *J. of Cell Biology*. 2017 Sep 4;216(9):2633-2644.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/28724527

Delage E, Cordero Cervantes D, Pénard E, Schmitt C, Syan S, Disanza A, Scita G, Zurzolo C, **Differential identity of Filopodia and Tunneling Nanotubes revealed by the opposite functions of actin regulatory complexes**, *Sci Rep* 2016 Dec;6:39632.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/28008977

Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, Olivo-Marin JC, Männel D, Zurzolo C, **Prions hijack tunnelling nanotubes for intercellular spread**, *Nat. Cell Biol*. 2009 Mar;11(3):328-36.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/19198598

Other (from our collaborators):

Kasthuri N, Hayworth KJ, Berger DR, Schalek RL, Conchello JA, Knowles-Barley S, Lee D, Vázquez-Reina A, Kaynig V, Jones TR, Roberts M, Morgan JL, Tapia JC, Seung HS, Roncal WG, Vogelstein JT, Burns R, Sussman DL, Priebe CE, Pfister H, Lichtman JW. **Saturated Reconstruction of a Volume of Neocortex**. *Cell*. 2015 Jul 30;162(3):648-61. doi: 10.1016/j.cell.2015.06.054.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/25018701

Livet J, Weissman TA, Kang H, Draft RW, Lu J, Bennis RA, Sanes JR, Lichtman JW. **Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system**. *Nature*. 2007 Nov 1;450(7166):56-62.
PubMed link: https://www.ncbi.nlm.nih.gov/pubmed/17972876

Scientific or technical background required for work program

Previous experience in neuroscience / neuroanatomy, graphic illustration, computer science, and/or cell biology is preferred but not mandatory. We are seeking motivated candidates who share our passion for science, are enthusiastic about learning new techniques, and are interested in combining multidisciplinary approaches at the forefront of neurobiology.

Title of the work program 27

Understanding the role of tunneling nanotubes between glioblastoma stem cells and astrocytes in the progression of Glioblastoma.

Description of the work program

Intra-tumoral heterogeneity plays a major role in tumor proliferation, invasion, as well as evasion to therapies. How cells **communicate in invasive cancers** has become a major research area and has been boosted by the discoveries of the role of exosomes and microvesicles. Moreover, Tunneling Nanotubes (TNTs), thin membranous open-ended channels connecting distant cells, have been observed connecting cancer cells and/or cancer and stromal cells in several aggressive forms of cancer, and points towards a novel mechanism for tumor molecular networking.

Glioblastoma (GBM) is the most aggressive primary brain tumor in adults and it is able to relapse despite chemo and radiotherapy. It is a heterogeneous disease with a complex **tumor microenvironment (TME)** and intra-tumor heterogeneity, which both play a major role in radiotherapy resistance. The GBM-TME consists of diverse cellular populations, including **astrocytes** and **glioblastoma cancer stem cells** (GSCs); GSCs have been shown to be the most resistant to treatments and to be at the origin of the relapse after treatment. It is known that cancer progression often depends on the interplay between tumor cells and neighbouring normal cells and it benefits from the selective conditions present in the TME, such as low concentration of oxygen (hypoxia). Furthermore, TNT-like structures form between GMB-derived cells, facilitate material exchange through them and correlate with a more aggressive phenotype. Our preliminary data utilizing 3D-tumor organoids, which better recapitulate tumor features, shows the formation of a network between the same type of GSCs, and between GSCs and murine astrocytes, previously observed *in vivo*. Furthermore, we observed mitochondria transfer via TNTs between GSCs and GSCs and astrocytes. *However, it remains unclear how this transfer of mitochondria has an effect on the metabolism of the acceptor population and its abilities to invade the surrounding environment.*

We hypothesize that an exchange of healthy mitochondria from astrocytes to GSCs and damaged mitochondria from GSCs to astrocytes, confer the GSCs an increase in the production of ATP using OSPHOs phosphorylation, evasion from the apoptosis induced by the damaged mitochondria and an increase in the invasiveness capacities.

Using tumor-organoids, our aim is to understand how cells behave at the *collective scale.* Microfluidic experiments will be used to generate an array of droplets containing GSCs and murine astrocytes to analyze the transfer of mitochondria between both populations, the variation in growth and morphology, the viablity of the cells and the invasivenes abilities of the acceptor population.

Presently, we are addressing this hypothesis by using different novel and classical techniques that the student will learn during the student's internship. For example, primary cell culture of murine astrocytes and GSCs, co-culture experiments of donor populations transduced with a lentiviral vector expressing a fluorescent marker of the inner mitochondrial membrane, the precursor of subunit 8 of human cytochrome c oxidase, fused to GFP or RFP (mitoGFP/RFP) with acceptor cell population expressing another fluorescent marker, like H2B-GFP/RFP. The student will learn to assess the metabolic profiling of the acceptor population using the Seahorse Bioscience extracellular flux (XF) analyzer to check the differences between normal (astrocytes) and cancerous cell lines (GSCs), effects of microenvironmental stress (hypoxia) and the ability of the transfer of mitochondria via TNT to alter the metabolic phenotypes of the acceptor population. He/She is going to have an important opportunity to learn new skills and competences in cell imaging (live-cell, fixed-cell, using different confocal and spinning disc microscopes in single cells and 3D cell organoid) will have the opportunity

to work under facilities that Institut Pasteur offers: the state-of-the-art equipment, seminars courses and the possibility of interact to international experts in the field of biological sciences. Moreover, working under interdisciplinary environment with physicists, neuroscientists and developmental biologists combining fundamental and translational research outside of his/her home country will be enriched him/she in order to achieve his/her perspective of career for example academic-based research after university.

Tutor/supervisor

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

1. Bhat S, Ljubojevic N, Zhu S, Fukuda M, Echard A, Zurzolo C. **Rab35 and its effectors promote tunneling nanotubes in neuronal cells**. *Sci. Rep.* (in press).
2. Vargas JY, Loria F, Wu Y-J, Córdova G, Nonaka T, Bellow S, Syan S, Hasegawa M, van Woerden GM, Trollet C, Zurzolo C. **The Wnt/Ca2+ pathway is involved in interneuronal communication mediated by tunneling nanotubes.** *EMBO J.* 2019 38:e101230.
3. Dilsizoglu Senol A, Pepe A, Grudina C, Sassoon N, Reiko U, Bousset L, Melki R, Piel J, Gugger M, Zurzolo C. **Effect of tolytoxin on tunneling nanotube formation and function,** *Sci. Rep.* 2019 9:5741.
4. Sartori-Rupp A, Cordero Cervantes D, Pepe A, Gousset K, Delage E, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, Zurzolo C. **Correlative cryo-electron microscopy reveals the structure of TNTs in neuronal cells,** *Nat. Commun.* 2019 10:342.
5. Zhu S, Bhat S, Syan S, Kuchitsu Y, Fukuda M, Zurzolo C. **Rab11a-Rab8a cascade regulates the formation of tunneling nanotubes through vesicle recycling,** *J. Cell. Sci.* 2018 131(19).
6. Loria F, Vargas JY, Bousset L, Syan S, Salles A, Melki R, Zurzolo C. **α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading,** *Acta Neuropathol.* 2017 134:789-808.
7. Delage E, Cervantes DC, Pénard E, Schmitt C, Syan S, Disanza A, Scita G, Zurzolo C. **Differential identity of Filopodia and Tunneling Nanotubes revealed by the opposite functions of actin regulatory complexes,** *Sci. Rep.* 2016 6:39632.
8. Abounit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R, Zurzolo C. **Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes,** *EMBO J.* 2016 35:2120-2138.
9. Abounit S, Delage E, Zurzolo C, **Identification and Characterization of Tunneling Nanotubes for Intercellular Trafficking,** *Curr. Protoc. Cell Biol.* 2015 67:12.10.1-21.

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

Scientific or technical background required for work program

We are looking for very motivated candidates interested in acquiring new skills and knowledge in cellular and molecular biology. Passion for science is essential, as well as to be enthusiastic about biological research. Previous experiences in cancer research and in cell/molecuilar biology and or microscopy is welcome, but not essential.

Title of the work program 28

Investigation of actin- and membrane-mediated pathways in the formation of tunneling nanotubes

Description of the work program

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

In standard cell culture, it is challenging to differentiate TNTs from visually similar filopodia, since no molecular marker yet exists. Utilizing micropatterning techniques to precisely control intercellular distances and cellular densities, we have identified physical parameters and distance thresholds over which the formation of functional TNTs is promoted. Our data shows that TNT formation between patterned cells occurs with the highest frequency at distances of 15−20 µm, and can be observed less frequently at distances of 30−40 µm; therefore, TNTs arise from specialized protrusions that reach longer distances than canonical filopodia that reach distances on the order of 5 µm. Additionally, our data suggests that there is a shift in the actin pool from branched to linear F-actin assemblies that correlates with more TNT-connected cells. For example, our preliminary data indicate that TNT-connected cells in basal conditions lack lamellipodia, and different treatments that tune this actin pool such as the Arp2/3 inhibitor CK666 and the formin agonist IMM01 all show an increase in the number of TNT-connected cells. ***However, it remains unclear which actin- and membrane-associating molecules are involved in the formation of functional TNTs.*** We previously demonstrated that a filopodia-promoting Cdc42/IRSp53/VASP network negatively regulates TNT formation and impairs TNT-mediated intercellular vesicle transfer in neuronal cells. Conversely, elevation of Eps8, an actin regulatory protein that inhibits the extension of filopodia in neurons, increases TNT formation in our cell model by facilitating F-actin bundling. ***We hypothesize that a switch in common actin regulatory pathways that shifts the cellular actin pool from branched to linear F-actin assemblies is critical in driving the formation of TNTs.*** Presently, we are addressing this hypothesis by two, parallel research directions. First, we are investigating how Eps8—a key actin regulator that leads to actin filament stabilization in TNTs—may work synergistically with membrane deforming and membrane curvature-sensitive proteins (inverted BAR domain proteins such as IRSp53 and IRTKS which have nM affinities for Eps8) in the formation of TNTs vs. filopodia. Second, we are assessing the role of the G-actin-binding protein, profilin, and how profilin may serve to redirect freely available G-actin in the cell away from

branched, Arp2/3-mediated actin networks and instead towards networks dominated by straight actin filament formation—most likely through Formin family proteins—necessary for TNTs.

During the student's internship, s/he will learn, for example, how to culture cells, to transfect them with expression vectors of the proteins of interest, to characterize direct protein-protein interactions by Western blot and immunoprecipitation, and to perform immunofluorescence labelling. Deep-UV micropatterning of fibronectin will be commonly used throughout the project to directly control the positioning of cells. In addition, the student will be trained to utilize both laser scanning and spinning disc confocal microscopes to acquire data on both fixed- and live-cell samples to characterize TNT formation and functionality. Additionally, the intern will gain experience in super-resolution microscopies (e.g., Live-SR confocal and structured illumination microscopy) to characterize protein localization within the cell and TNTs, and fluorescent recovery after photobleaching (FRAP) measurements to assess the development of actin within the TNTs. Interns will also gain computational expertise utilizing ImageJ/Fiji and Icy for image-based analysis. Finally, the student will be trained to use cell cytometry methods (e.g., Amnis ImageStream Mark II) in order to evaluate the functionality of these TNTs by measuring the transfer of vesicles, or mitochondria, between donor and acceptor cell populations.

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

Tutor/supervisor

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

1. Bhat S, Ljubojevic N, Zhu S, Fukuda M, Echard A, Zurzolo C. **Rab35 and its effectors promote tunneling nanotubes in neuronal cells**. *Sci. Rep.* (in press).

2. Vargas JY, Loria F, Wu Y-J, Córdova G, Nonaka T, Bellow S, Syan S, Hasegawa M, van Woerden GM, Trollet C, Zurzolo C. **The Wnt/Ca2+ pathway is involved in interneuronal communication mediated by tunneling nanotubes.** *EMBO J.* 2019 38:e101230.
3. Dilsizoglu Senol A, Pepe A, Grudina C, Sassoon N, Reiko U, Bousset L, Melki R, Piel J, Gugger M, Zurzolo C. **Effect of tolytoxin on tunneling nanotube formation and function,** *Sci. Rep.* 2019 9:5741.
4. Sartori-Rupp A, Cordero Cervantes D, Pepe A, Gousset K, Delage E, Corroyer-Dulmont S, Schmitt C, Krijnse-Locker J, Zurzolo C. **Correlative cryo-electron microscopy reveals the structure of TNTs in neuronal cells,** *Nat. Commun.* 2019 10:342.
5. Zhu S, Bhat S, Syan S, Kuchitsu Y, Fukuda M, Zurzolo C. **Rab11a-Rab8a cascade regulates the formation of tunneling nanotubes through vesicle recycling,** *J. Cell. Sci.* 2018 131(19).
6. Loria F, Vargas JY, Bousset L, Syan S, Salles A, Melki R, Zurzolo C. **α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading,** *Acta Neuropathol.* 2017 134:789-808.
7. Delage E, Cervantes DC, Pénard E, Schmitt C, Syan S, Disanza A, Scita G, Zurzolo C. **Differential identity of Filopodia and Tunneling Nanotubes revealed by the opposite functions of actin regulatory complexes,** *Sci. Rep.* 2016 6:39632.
8. Abounit S, Bousset L, Loria F, Zhu S, de Chaumont F, Pieri L, Olivo-Marin JC, Melki R, Zurzolo C. **Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes,** *EMBO J.* 2016 35:2120-2138.
9. Abounit S, Delage E, Zurzolo C, **Identification and Characterization of Tunneling Nanotubes for Intercellular Trafficking,** *Curr. Protoc. Cell Biol.* 2015 67:12.10.1-21.
10. Gousset K, Marzo L, Commere PH, Zurzolo C. **Myo10 is a key regulator of TNT formation in neuronal cells,** *J. Cell. Sci.* 2013 126:4424-35.
11. Costanzo M, Abounit S, Marzo L, Danckaert A, Chamoun Z, Roux P, Zurzolo C. **Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes,** *J. Cell. Sci.* 2013 126:3678-85.
12. Gousset K, Schiff E, Langevin C, Marijanovic Z, Caputo A, Browman DT, Chenouard N, de Chaumont F, Martino A, Enninga J, Olivo-Marin JC, Männel D, Zurzolo C. **Prions hijack tunnelling nanotubes for intercellular spread,** *Nat. Cell Biol.* 2009 11:328-36.

Scientific or technical background required for work program

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

Title of the work program 29

Structural studies of histidine kinases involved in enterobacteria drug resistance

Description of the work program

Sensor histidine kinases are membrane-anchored receptors that meditate many adaptive responses in bacteria, including pathogens resistance to antimicrobial drugs. We are interested in revealing the mechanisms of signal transduction for this important family of sensor kinases. In particular, we want to understand how the input signal propagates across the membrane to modulate the different catalytic activities of the receptor.
This project aims to characterize at the molecular level histidine kinase receptors involved in antibiotic resistance and virulence regulation. During the internship, the student will participate in the expression and purification of selected sensor kinases from enterobacteria pathogens, as well as in its structural characterisation by X-ray crystallography and/or cryo-electron microscopy.

Tutor/supervisor

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

1: Jacob-Dubuisson F, Mechaly A, Betton JM, Antoine R. Structural insights into the signalling mechanisms of two-component systems. Nat Rev Microbiol. 2018 Oct;16(10):585-593. doi: 10.1038/s41579-018-0055-7. PMID: 30008469.

2: Mechaly AE, Soto Diaz S, Sassoon N, Buschiazzo A, Betton JM, Alzari PM. Structural Coupling between Autokinase and Phosphotransferase Reactions in a Bacterial Histidine Kinase. Structure. 2017 Jun 6;25(6):939-944.e3. doi: 10.1016/j.str.2017.04.011. Epub 2017 May 25. PMID: 28552574.

3: Mechaly AE, Sassoon N, Betton JM, Alzari PM. Segmental helical motions and dynamical asymmetry modulate histidine kinase autophosphorylation. PLoS Biol. 2014 Jan 28;12(1):e1001776. doi: 10.1371/journal.pbio.1001776. PMID: 24492262; PMCID: PMC3904827.

Scientific or technical background required for work program

Some knowledge on basic molecular biology and protein biochemistry techniques.

Title of the work program 30

Dynamic study of serotonin receptors by genetically encoded fluorescent probes.

Description of the work program

Serotonin ion channels (5-HT_3R) are transmembrane proteins belonging to the superfamily of ligand gated ion channels (LGICs). They assemble as pentameric assemblies of sub-units and are parts of the family of Cys-loop receptors, responsible namely for the fast-synaptic communication between neurons[1]. Disorders in the action of 5-HT_3R are linked with several pathological conditions such as nausea, psychiatric disorders, depression, schizophrenia, Parkinson's disease and irritable bowel[2]. Understanding precisely the functioning of these channels, to design more potent and more selective drugs, is then of particular importance.

Key discoveries concerning the functioning of these proteins has been made by the establishment of atomic structures (obtained either by X-Ray crystallography[3] or by Cryo-EM[4]). Unfortunately, these techniques suffer from their inherent limitations (truncations, mutations, use of surfactants, needs of removing the protein from its membrane environment) and depict a static picture of the conformation. In order to study dynamic molecular motions decisive for the activation and the functioning of these receptors, we have implemented the Voltage-Clamp Fluorometry (VCF) technique in our lab[5]. This strategy allows to record the electric activity of the full-length channel correctly expressed into an oocyte membrane simultaneously with the observation of molecular motions that are reported by a change in the fluorescence intensity. By engineering pairs of fluorophore/quencher into the channel, distance measurements can be made in areas of the protein that are critical for the activation. This engineering is therefore limited to extracellular, solvent-accessible positions for the labelling with classic fluorophores. To overcome this limitation, genetic incorporation of fluorescent amino acids has been developed and used to perform VCF experiment in other pentameric LGICs[6].

The objective is to perform VCF experiments with fluorescent genetically-encoded amino acids incorporated into serotonin receptors to study the transmembrane and intracellular parts of the 5-HT_3R channel. The motions observed will then be interpreted in term of models of activation of serotonin receptors. The student will be supervised by a post-doctoral researcher working on the same system.

[1] Nemecz, Á., Prevost, M. S., Menny, A. & Corringer, P.-J. Emerging Molecular Mechanisms of Signal Transduction in Pentameric Ligand-Gated Ion Channels. *Neuron* **90**, 452–470 (2016).
[2] Thompson, A. J., Lester, H. A. & Lummis, S. C. R. The structural basis of function in Cys-loop receptors. *Quart. Rev. Biophys.* **43**, 449–499 (2010).
[3] Hassaine, G. *et al.* X-ray structure of the mouse serotonin 5-HT3 receptor. *Nature* **512**, 276–281 (2014).
[4] Polovinkin, L. *et al.* Conformational transitions of the serotonin 5-HT 3 receptor. *Nature* **563**, 275–279 (2018).
[5] Menny, A. *et al.* Identification of a pre-active conformation of a pentameric channel receptor. *eLife* **6**, e23955 (2017).

[6] Soh, M. S., Estrada-Mondragon, A., Durisic, N., Keramidas, A. & Lynch, J. W. Probing the Structural Mechanism of Partial Agonism in Glycine Receptors Using the Fluorescent Artificial Amino Acid, ANAP. *ACS Chem. Biol.* **12**, 805–813 (2017).

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

Nemecz, Á., Prevost, M. S., Menny, A. & Corringer, P.-J. Emerging Molecular Mechanisms of Signal Transduction in Pentameric Ligand-Gated Ion Channels. *Neuron* **90**, 452–470 (2016).

Menny, A. *et al.* Identification of a pre-active conformation of a pentameric channel receptor. *eLife* **6**, e23955 (2017).

Scientific or technical background required for work program

Background in biochemistry and/or biophysics

Title of the work program 31

Role of microRNAs in the IFN-dependent modulation of the human T cell adaptive immune response

Description of the work program

We study the immunomodulatory activity of type I interferon family (IFNα/β) on the human T cell immune response in healthy donors and in patients with multiple sclerosis (MS). This chronic autoimmune and inflammatory disease targets the central nervous system and leads to axonal demyelination, neurodegeneration and gradual physical disabilities. The most common form of the disease is relapsing-remitting (RRMS), which is commonly treated by IFNβ as a first-line therapy. Our major goal is to uncover cellular and molecular immune signatures that could help to determine disease and treatment response biomarkers. Based on RNA-seq data and recent results of the laboratory, we have identified microRNAs (miRNAs) candidates that potentially regulate the expression of the anti-inflammatory cytokine IL10 and the IFN response. MiRNAs are small non-coding RNAs that bind to specific sites in the 3' untranslated region of messenger RNAs, resulting in cleavage, destabilization or reduced translation of mRNAs, and thereby gene silencing or decreased gene expression. A number of our miRNAs candidates are predicted to be distinctly activated in different immune cell types and according to the activation state. Expression miRNAs will be studied in human blood cells and in purified T cells from healthy donors and RRMS patients at different stages of the disease and/or under IFNβ treatment. Mechanistic studies may be conducted in order to assess the impact of specific miRNAs in the modulation of the T cell response by IFNβ.

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

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

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

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

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

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

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

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

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

Scientific or technical background required for work program

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

Erasmus+

Institut Pasteur 2020-2021

Title of the work program 32

Molecular mechanisms linking mitochondrial dysfunction with altered bacterial infection

Description of the work program

Mitochondrial diseases are relatively common heritable diseases (>1/5000), caused by mutations in mitochondrial proteins that lead to a respiratory defect. These patients suffer from recurrent infections, which can be life-threatening or lead to disease progression. Few studies have addressed the role of mitochondrial respiration in infection; we established cellular models where we manipulated the respiratory activity and challenged them with the intracellular bacterium *L.monocytogenes*. Our results indicate that infection is favoured in cells with impaired respiration. In this host-pathogen project, we want to evaluate whether the respiratory defects of our models impact the innate immune response and how this affects infection by *L.monocytogenes* and other intracellular bacteria.

Tutor/supervisor

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

Listeria monocytogenes Exploits Mitochondrial Contact Site and Cristae Organizing System Complex Subunit Mic10 To Promote Mitochondrial Fragmentation and Cellular Infection
Carvalho F, Spier, A, Chaze T, Matondo M, Cossart, P[#] and Stavru, F[#] ([#] corresponding authors)
mBio. 2020 4;11(1). pii: e03171-19. doi: 10.1128/mBio.03171-19.

Interaction between intracellular bacterial pathogens and host cell mitochondria
Spier, A, Stavru, F and Cossart, P
Microbiol Spectr. 2019 Mar;7(2)
doi: 10.1128/microbiolspec.BAI-0016-2019

Scientific or technical background required for work program

We are seeking for an enthusiastic student. Scientific background in cell biology is needed, as well as ability to work in a team and organizational skills. Experience with cell biological methods (cell culture, transfection, confocal microscopy) and standard biochemistry/molecular biology is preferable, but not essential.