Projects Fall 2017

Erasmus+

Institut Pasteur 2017-2018

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 26 projects for Erasmus internships (see following pages). Students can 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 550 euros/month is paid by the host lab
- **Others** (some institutions offer a round trip plane ticket): .....

Title of the work program 1

Role of intermediate filaments in mechanotransduction during cell migration

Description of the work program

The cell cytoskeleton is mainly composed of three distinct filamentous networks: actin microfilaments, microtubules and intermediate filaments (IFs). Until now, actin and microtubule functions have been extensively studied, but much less is known about the role of IFs. Several lines of evidence point to a role of IFs in cell mechanics and cell migration (Leduc and Etienne-Manneville, 2015). Changes in the composition and the network organization of IFs occur during cell migration and participate in tumor cell invasion. Using in vitro models of astrocyte and glioblastoma cell migration we have demonstrated that IFs control cell polarity and nucleus positioning (Dupin and Etienne-Manneville, 2011; Dupin et al., 2011). Cell interaction with the extracellular matrix triggers signaling cascades leading to IF rearrangements (Leduc & Etienne-Manneville, J Cell Biol 2017). Our hypothesis is that the physical properties of the cell microenvironment change IF organization to change nuclear shape, orientation and position and to ultimately affect nuclear architecture and gene expression. The general goal of this project is to determine how IFs respond to the physical properties of the cell microenvironment.
The project will consist in determining the impact of substrate rigidity on the organization and mechanical functions of intermediate filaments during cell migration.
- Substrates of controlled rigidity and composition will be used to assess intermediate filament organization in immobile cells plated on micropatterns to control cell shape and in migrating cells. We expect to observe an influence of the substrate rigidity on the polymerization of intermediate filaments and on their spreading in the cell cytoplasm.
-The involvement of mechanosensing proteins (Talin, vinculin) at focal adhesions will be tested using siRNA or specific constructs
This project will mainly require cell culture, micromanipulation of substrates (controlling rigidity and micropatterning), microscopy and videomicroscopy and cutting edge image analysis.

Tutor/supervisor

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

Most relevant publications (2011-2017)

1. Leduc C, Etienne-Manneville S. *Regulation of microtubule-associated motors drives the polarization of the intermediate filament network*. **J. Cell Biol.** 2017. Jun 5;216(6):1689-1703.
2. Mayor R, Etienne-Manneville S. *The front and rear of collective cell migration.* **Nat Rev Mol Cell Biol.** 2016 Feb;17(2):97-109.
3. Dupin I, Elric J, Etienne-Manneville S. *Adhesive micropatterns to study intermediate filament function in nuclear positioning*. **Curr Protoc Cell Biol.** 2015 Mar 2;66:13.7.1-13.7.19.
4. Leduc C, Etienne-Manneville S. *Intermediate filaments in cell migration and invasion: the unusual suspects*. **Curr Opin Cell Biol.** 2015 Feb;32:102-12
5. Boëda B, Etienne-Manneville S. Spectrin binding motifs regulate Scribble cortical dynamics and polarity function. **Elife.** 2015 Feb 9;4
6. S. Etienne-Manneville. 2014. *Neighborly relations during collective migration.* **Curr Opin Cell Biol.** Jul 2;30C:51-59.
7. F. Peglion, F. Llense, S. Etienne-Manneville. 2014. *Adherens junction treadmilling during collective migration.* **Nat. Cell Biol**. Jul; 16(7):639-51.
8. S. Etienne-Manneville. 2013. *Centrosome, microtubules and polarized cell migration*. in **Ann Rev. Cell Dev Biol**., Volume 29; October 6
9. Y. Sakamoto, B. Boëda, S. Etienne-Manneville. 2013. *APC binds intermediate filaments and is required for their reogranization during cell migration.* **J. Cell Biol.** Feb 4;200(3):249-58.
   ↳Selected for the production of a biosight.
10. E. Camand, F. Peglion, N. Osmani, Sanson M., S. Etienne-Manneville. 2012. *N-cadherin expression level modulates integrin-mediated polarity and strongly impacts on the speed and directionality of glial cell migration.* **J. Cell Sci.** Feb 15;125(Pt 4):844-57.
11. I. Dupin, Y. Sakamoto, S. Etienne-Manneville. 2011. *Cytoplasmic intermediate filaments mediate actin-driven nucleus positioning.* **J. Cell Sci.** Mar 15;124(Pt 6):865-72.

Scientific or technical background required for work program

We are looking for highly motivated student. Experience in either cell biology, microscopy, mechanobiology would be a plus. This project may be extended to the PhD on how IFs responses to the physical properties of the cell microenvironment affect nuclear positioning, rotation and shape and how IFs affect nuclear organization

Title of the work program 2

Genome Engineering of *Vibrio cholerae* phage

Description of the work program

Bacteriophages are an underexploited source of modified nucleotides. These modified bases are thought to be a defense mechanism developped by the bacteriophages to escape to the host restriction system. They might also be required for their replication.

We focused our work on a *Vibrio cholerae* bacteriophage in addition to two actinobacteriophages lytic for two Gram+ bacteria *Gordonia terrae* and *Arthrobacter* sp. These phages were chosen because their genome contain several ORFs that code for enzymes susceptible to interfere with the host metabolism (deoxyuridine triphosphatase, adenylosuccinate synthetase....) and enzymes involved in their replication (DNA primase, helicase, DNA polymerase,...)

We propose to engineer these bacteriophages using CRISPR-Cas9 as described by Lemay et al (2017 ACS Synth. Biol., Article ASAP DOI: 10.1021/acssynbio.6b00388). We will adapt this method to the bacteria mentionned above in order to modify the differnet phages by deleting the ORFs of interest. This will allow to study the role of essential phage genes in vivo. The *Vibrio cholerae* phage engineering will be done in collaboration with Pr D. Mazel Unite de Plasticite du Genome bacterien, Institut Pasteur.

Tutor/supervisor

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

- Andrade WA, Firon A, Schmidt T, Hornung V, Fitzgerald KA, Kurt-Jones EA, Trieu-Cuot P, Golenbock DT, **Kaminski PA**. Group B Streptococcus Degrades Cyclic-di-AMP to Modulate STING-Dependent Type I Interferon Production. Cell Host Microbe. 2016 Jul 13;20(1):49-59.
- Ye W, Paul D, Gao L, Seckute J, Sangaiah R, Jayaraj K, Zhang Z, **Kaminski PA**,

Ealick SE, Gold A, Ball LM. Ethenoguanines undergo glycosylation by nucleoside 2'-deoxyribosyltransferases at non-natural sites. PLoS One. 2014 Dec 18;9(12):e115082.

- Firon A, Dinis M, Raynal B, Poyart C, Trieu-Cuot P, **Kaminski PA**. Extracellular nucleotide catabolism by the Group B Streptococcus ectonucleotidase NudP increases bacterial survival in blood. J Biol Chem. 2014 Feb 28;289(9):5479-89.
- Sikowitz MD, Cooper LE, Begley TP, **Kaminski PA**, Ealick SE. Reversal of the substrate specificity of CMP N-glycosidase to dCMP. Biochemistry. 2013 Jun 11;52(23):4037-47.
- Amiable C, Pochet S, Padilla A, Labesse G, **Kaminski PA**. N (6)-substituted AMPs inhibit mammalian deoxynucleotide N-hydrolase DNPH1. PLoS One. 2013 Nov 19;8(11):e80755.

Scientific or technical background required for work program

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

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

Title of the work program 3

Towards the identification of disease mechanisms and rescuable therapeutic targets for progressive hearing deficits

Description of the work program

The hearing loss greatly impedes communication, leading to social isolation, depression and reduced physical and cognitive function. Deafness is the most frequent cause of inherited sensory deficits in humans, and the number of hearing-impaired patients dramatically increases with ageing, especially in exposed environments. According to the World Health Organization's estimates (http://www.who.int), prevalence of hearing impairment will increase from > 360 million in 2011 to over 1 billion individual by 2050. These major sensory deficits still represent unmet medical needs, since treatment options are largely missing and the economic burden on healthcare systems worldwide keeps increasing. Today, about 100 deafness genes have been identified, and many more are yet to be identified. Albeit tremendous progress has been made on how the hearing organ develops, and on the discovery of genes causing congenital/early onset profound hearing loss, we know very little on how adult hearing organ maintains its normal activity and the mechanisms involved in progressive, late-onset hearing impairments. Through this project, we expect to identify new key players in sound processing by the auditory hair cells, which is critical for the precise encoding of acoustic information enabling normal speech discrimination.

Ongoing work on two deaf mutant mice pinpoints the key role of 2 tetraspan proteins (integral membrane proteins with 4 trans-membrane domains) in sound induced transduction by the auditory hair cells, and their signal transmission to the brain. New cell-specific mutant mice are being produced and this project' aims are designed to unravel these proteins' functions, and follow-up a thorough and accurate physiological, cellular, and molecular phenotyping of these disease animal models. Because genetic as well as non-genetic/environmental risks are know to impact individuals susceptibility to a disease, pathogenesis severity and its rate of progression, this project is designed to unveil possible impact of noise exposure, and/or age on the progression of hearing impairment.

Some of our specific aims, which involve lab-internal and external collaborations, are:

(i) **Hearing protein-protein interaction (PPI) networks**: Identify binding partners of the deaf causal genes, aiming to map sequence of events leading to hearing loss. After *in silico* analyses of causal genes' molecular interacting partners (identified through candidate and yeast-two hybrid (Y2H) screen approaches; Y2H data already available for 2 new deaf genes), biochemical and cell biology experiments (varying according to gene identity) will be used *in vitro* and *in cellulo* to confirm selected PPI hits, with the challenge to decipher the ways a confirmed network drives given phenotype *in vivo*.

(ii) **Hearing abilities of deaf mutant mice:** Audiometric tests will be performed to determine for each mouse model the extents and peculiarities of the observed hearing impairment (onset of hearing impairment, constant or progressive loss, sensitivity to all frequencies, and cell types involved…).

(iii) **Morphological and molecular phenotyping:** light confocal (e.g. tissue clearing combined with 3D rendering to monitor neuronal state for tissue-level map) and electron microscopy imaging techniques will be used to determine subcellular-specific patterns that accurately reflect in vivo disease cell-specific states; comparative analyses in wild-type and deaf mice will be performed under normal and challenged environmental conditions. Appropriate subcellular markers (for the sound-receptive structure, the hair bundle, and for the pre- and post-synaptic regions of hair cells) will be used to document the structural alterations, and possible protein mislocalization in mutant models.

The outcome of detected abnormalities will be also monitored, upon viral mediated gene therapy approaches in the deaf mutant mice to prevent and/or correct the hearing. By determining

early, intermediate and terminal stages of the disease, the speed and outcome of hearing deterioration the findings will lead back to clinical discoveries in diagnosis, phenotyping, and open up new horizons for innovative therapeutic strategies to treat inner ear disorders.

Tutor/supervisor

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

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. *Co-senior and corresponding authors.

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 Medicine “Recommended” selection.

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 Medicine “Recommended” selection; # cover article.

Potter P, Bowl M, (+ 44 authors MRC Harwell ageing screen consortium), **El-Amraoui A**, Petit C, Acevedo-Arozena A, Nolan P, Cox R, Mallon AM, & Brown SD. **(2016)** Novel gene function revealed by mouse mutagenesis screens for models of age-related disease. ***Nat. Comm.*** 7:12444.

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.

Papal S, Cortese M, Legendre K, Sorusch N, Dragavon J, Sahly I, Shorte S, Wolfrum U, Petit C, **El-Amraoui A*. (2013)** The giant spectrin βV couples the molecular motors to phototransduction and Usher syndrome type I proteins along their trafficking route. ***Hum. Mol. Genet.*** 22, 3773-3788.
* Corresponding author.

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 Medicine “Recommended” selection.

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-mentioned approaches will be considered**

Title of the work program 4

Structural study of a pentameric ligand gated ion channel

Description of the work program

Our major subject concerns pentameric ligand gated ion channels (pLGICs, including nicotinic, $GABA_A$ and glycine receptors) that mediate neuronal communication. pLGICs are involved in many diseases, including nicotinic addiction, epilepsy, neurodegenerative and psychiatric diseases such as Alzheimer and Parkinson diseases. They are the target of important classes of therapeutic and addictive drugs including general anesthetics (targeting principally $GABA_A$ receptors), anxiolytics (benzodiazepines that are portentiators of the $GABA_A$ receptors), anti-vomiting compounds (antagonists of $5HT_3$ receptor) and anti-smoking compounds (targetting nAChRs). Therefore, understanding the molecular mechanisms involved in functioning of these channel-receptors, as well as their regulation/deregulation by therapeutic and addictive drugs is currently a matter of intense research activity by academia and industry.
Following the discovery of a bacterial ancestor of pLGICs called GLIC in our lab, we collected a set of structural (using X-ray crystallography and fluorescence) and functional (using electrophysiology and surface plasmon resonance) data allowing us to propose an advanced mechanism of activation for a member of this family of channels.
Our group headed by Nicolas Wolff recently joined the team and has key expertise in biophysics (NMR, X-ray crystallography, SAXS, …). Our project aims at further understanding the fundamental allosteric mechanisms mediating the signal transduction of pLGICs. Using the model system GLIC, we will develop NMR and X-ray crystallography experiments, to elucidate the structural dynamics of this protein. Indeed, pLGICs are intrinsically dynamic proteins, in constant equilibrium between allosteric states.
We will improve the yield of expression of GLIC in *E. coli*, in order to perform NMR experiments. NMR spectroscopy coupled to specific labelling is well-suited to characterize transiently populated conformations as well as the transitions between different conformations in solution. We will label and then perform ^{19}F and ^{13}C NMR spectroscopy of fluorine-, $^{13}CH_3$-ε-methionines and ^{13}C-dimethylated-lysines labeled GLIC expressed in appropriate *E. coli* strains and medium. In addition, selected GLIC mutants will be investigated by X-ray crystallography to search for new conformations.
The whole set of data will be compared to the various structures of GLIC to propose a global transition pathway for activation and desensitization. It will be also useful to guide and interpret molecular dynamics simulations that are actively investigated on GLIC.

Tutor/supervisor

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

Prevost M.S., Sauguet L., Nury H., Van Renterghem C., Huon C., Poitevin F., Baaden M., Delarue M., Corringer, P.J. (2012) A locally closed conformation of a bacterial pentameric proton-gated ion channel. *Nat. Struct. Mol. Biol.* 19:642-9.

Prevost M.S., Moraga-Cid G., Van Renterghem C., Edelstein J, Changeux JP*, Corringer P.J.* (2013) Intermediate closed state for glycine receptor function revealed by cysteine cross-linking. *Proc. Natl. Acad. Sci. USA* 110(42):17113-8.

Maisonneuve P, Caillet-Saguy C, Raynal B, Gilquin B, Chaffotte A, Pérez J, Zinn-Justin S, Delepierre M, Buc H, Cordier F, Wolff N. (2014) Regulation of the catalytic activity of the human phosphatase PTPN4 by its PDZ domain. FEBS J. 281(21):4852-65.

Moraga-Cid G., Sauguet L., Huon C., Malherbe L., Girard-Blanc C., Petres S., Murail S., Taly A., Baaden M. Delarue M., Corringer P.J. (2015) Allosteric and hyperekplexic mutant phenotypes investigated on an α_1 glycine receptor transmembrane structure. *Proc. Natl. Acad. Sci. USA* 112:2865-2870.

Maisonneuve P, Caillet-Saguy C, Vaney MC, Bibi-Zainab E, Sawyer K, Raynal B, Haouz A, Delepierre M, Lafon M, Cordier F, Wolff N. (2016) Molecular Basis of the Interaction of the Human Protein Tyrosine Phosphatase Non-receptor Type 4 (PTPN4) with the Mitogen-activated Protein Kinase p38γ. J Biol Chem. 291(32):16699-708.

Menny A., Lefebvre S.N., Schmidpeter P., Drège E., Fourati Z., Delarue M., Edelstein S.J., Nimigean C.M., Joseph D., Corringer P.J. (2017) Identification of a pre-active conformation of a pentameric channel receptor. *eLife* 10.7554/eLife.23955.

Scientific or technical background required for work program

Methodologies:

The student mainly use biochemical and biophysical approaches applied to proteins:

- Expression and purification of recombinant proteins expressed in *E. coli*
- Biophysical experiments

Prerequisite:

- General knowledge in protein biochemistry
- Interest in Structural Biology

The student will produce different mutants of GLIC. He will conduct biochemical, biophysics and structural studies with these mutants. Crystallogenesis tests will be performed to obtain structures and proteins will be labeled with isotopes to study their behavior in solution by NMR.

These approaches benefit from the expertise of team members and facilities of the platforms on the campus.

Title of the work program 5

Investigation of microRNAs regulating type I interferon signaling

Description of the work program

Type I interferons (IFN-I) is a well-known conserved cytokine family that contributes to innate defense against viruses and influences the development of adaptive antimicrobial and antitumor immunity. IFN-I regulate homeostasis, differentiation and function of a variety of cell lineages. This pleiotropic action requires the rapid and transient transcriptional induction of hundreds of IFN-stimulated genes. Multiple layers of regulation set the timing and the magnitude of IFN-I action. If the production of IFN-I or the response to IFN-I is dysregulated, pathogenic processes can occur, as in several autoimmune diseases and in the rare genetic disorders called interferonopathies.

Our laboratory is interested in dissecting the regulation of IFN-I signaling in humans. Recently we have focused on USP18, an IFN-stimulated gene acting as key negative feedback regulator that abrogates signaling. With the view that the level of USP18 is critically controlled in a cell- and context-specific manner, we are exploring the possibility of new layers of regulation at the mRNA level. Through stringent bioinformatics algorithms we have selected a number of microRNAs that are predicted to target with high confidence the 3'UTR of *USP18*. We have also identified a predicted LincRNA (long intergenic non-coding RNA) that may regulate USP18 through a sponging mechanism.

The candidate will investigate: 1) the impact of chosen miRNAs on basal and induced USP18 (mRNA and protein) by overexpression and inhibition in model cell lines; 2) the possibility that the miRNAs act upon IFN-I signaling and bioactivities; 3) the expression and regulation of these miRNAs in various immune cell types.

Tutor/supervisor

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

Most relevant publications (2011-2017)

1. Govender U, Corre B, Bourdache Y, Pellegrini S, Michel F. 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.* 101:1181
2. Arimoto KI, Löchte S, Stoner SA, Burkart C, Zhang Y, Miyauchi S, Wilmes S, Fan JB, Heinisch JJ, Li Z, Yan M, Pellegrini S, Colland F, Piehler J, Zhang DE. 2017. STAT2 is an essential adaptor in USP18-mediated suppression of type I interferon signaling. *Nat Struct Mol Biol.* 24:279-289

3. Meuwissen ME, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, Li Z, van Unen L, Heijsman D, Goldmann T, Lequin MH, *et al*, Prinz M, Crow YJ, Verheijen FW, Pellegrini S, Bogunovic D and Mancini GM. Human USP18 deficiency underlies type 1 interferonophathy leading to severe pseudo-TORCH syndrome. *J. Exp. Med.*, 213:1163
4. Speer SD, Li Z, Buta S, Payelle-Brogard B, Qian L, Vigant F, Rubino E, Gardner TJ, Wedeking T, Hermann M, Duehr J, Sanal O, Tezcan I, Mansouri N, Tabarsi P, Mansouri D, Francois-Newton V, Daussy CF, Rodriguez MR, Lenschow DJ, Freiberg AN, Tortorella D, Piehler J, Lee B, Garcia-Sastre A, Pellegrini S* and Dusan Bogunovic*. ISG15 deficiency and increased viral resistance in humans but not mice. *Nature Communications*, 7:11496
5. Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer S, *et al*, Boisson-Dupuis S, Crow YJ, Casanova J-L and Pellegrini S. 2015. Human intracellular ISG15 prevents IFN-alpha-beta over-amplification and auto-inflammation. *Nature*, 517:89-93
6. Wilmes S, Beutel O, Li Z, Francois-Newton V, Richter CP, Janning D, Kroll C, Hanhart P, Hötte K, You C, Uzé G, Pellegrini S, Piehler J. 2015. Receptor dimerization dynamics as regulatory valve for plasticity of type I interferon signalling. *J Cell Biol* 209:579-93
7. Li Z, Gakovic M, Ragimbeau J, Eloranta ML, Rönnblom L, Michel F and Pellegrini S. 2013. Two rare disease-associated TYK2 variants are catalytically impaired but signaling competent. *J. Immunol*. 190:2335-4
8. Francois-Newton V, Livingstone M, Payelle-Brogard B, Uzé G and Pellegrini S. 2012. USP18 establishes the transcriptional and anti-proliferative interferon alpha-beta differential. *Biochem J*. 446:509-16
9. Francois-Newton, V., G. de Freitas Almeida, B. Payelle-Brogard, D. Monneron, L. Pichard-Garcia, J. Piehler, S. Pellegrini*, and G. Uzé*. 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:e22200.

Scientific or technical background required for work program

The student will be committed and motivated. She/he will have a good theoretical background and some technical experience in cellular and molecular biology and be familiar with cell signaling mechanisms. A good knowledge in basic immunology is highly recommended.

Techniques : molecular biology ; protein structure-function analyses ; Jak/Stat signaling.

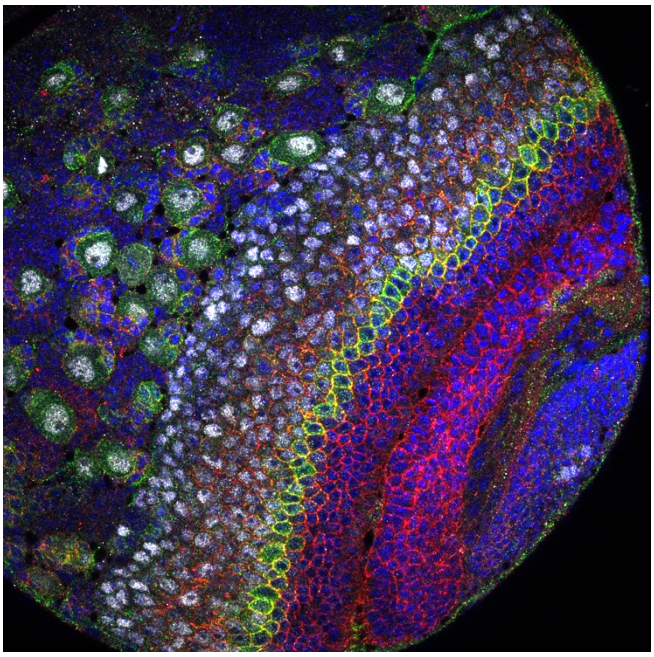
Title of the work program 6

Cell shape and polarity changes in emerging Neural Stem Cells in the fruit fly brain

Description of the work program

Neural stem cells (NSCs) give rise to the neurons and glia that will populate the adult brain. As such neurogenesisis is a tightly orchestrated process that must balance proliferation and differentiation choices such that the correct number and lineage specificity of progeny are generated to ensure tissue homeostasis. Stem cells in many tissues, including the nervous system, initially undergo an expansion phase when they divide symmetrically to expand the pool of tissue specific precursors. From flies to mammals, symmetrically dividing neural precursors are comprised of sheets of neuroepithelial cells (NECs). Later in development signalling cues initiate a switch to asymmetric division, where one daughter cell will begin to differentiate into a terminal cell fate, such as a neuron.

In order to study the mechanics on an individual cell level that underpin the coordinated switch from symmetric to asymmetric division in the nervous system we use the *Drosophila* optic lobe as an in *vivo* model. During larval development in fruit flies a proneural wave sweeps the optic lobe neuroepithelium converting symmetrically dividing NECs into asymmetrically dividing NSCs in a region called the transition zone. While the signals and transcription factors that drive proneural wave progression are well known, the cellular changes underlying this switch are less well understood. In order for a NEC to become a NSC, multiple events need to occur, including changes to cell shape, cell-cell adhesion, and polarity. In this project we aim to identify the progression of events that lead to down regulation of apical polarity proteins and disassembly of adherens junctions in the transition zone. We also aim to identify the upstream regulators of these dynamic cellular changes. This will be elucidated using a combination of live imaging and *in vivo* genetic manipulation.



***Drosophila* optic lobe:**

- Neural Stem Cells (white nuclei)
- Neuroepithelium (Adherens junctions in red)
- Transition zone (specific regulator, green)
- all nuclei (blue)

Tutor/supervisor

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

L. Couturier, N. Vodovar and F. Schweisguth (2012) Endoyctosis by Numb breaks Notch symmetry at cytokinesis. Nature Cell Biology, 14, 131-9

S. Chanet and F. Schweisguth (2012) Regulation of epithelial polarity by the E3 ubiquitin ligase Neuralized and the Bearded inhibitors in *Drosophila*. Nature Cell Biology, 14, 467-76

L. Couturier, K. Mazouni and F. Schweisguth (2013) Numb localizes at endosomes and controls the endosomal sorting of Notch after asymmetric division in Drosophila. Current Biology, 23, 588-93

L. Couturier, M. Trylinski, K. Mazouni, L. Darnet and F. Schweisguth (2014) A fluorescent tagging approach in Drosophila reveals late endosomal trafficking of Notch and Sanpodo. The Journal of Cell Biology, 3, 351-63

C. Besson, F. Bernard, F. Corson, H. Rouault, E. Reynaud, A. Keder, K. Mazouni and F. Schweisguth (2015) Planar Cell Polarity breaks the symmetry of PAR protein distribution prior to mitosis in *Drosophila* Sensory Organ Precursor cells. Current Biology, 25, 1104-10

S. Pontier and F. Schweisguth (2015) A *Wolbachia*-sensitive communication between male and female pupae controls gamete compatibility in *Drosophila*. Current Biology, 25, 2339-48

G. Perez-Mockus, V. Roca, K. Mazouni and F. Schweisguth (2017) Neuralized regulates Crumbs endocytosis and epithelium morphogenesis via specific Stardust isoforms. The Journal of Cell Biology, 205, 1405-20

F. Corson, L. Couturier, H. Rouault, K. Mazouni and F. Schweisguth (2017) Self-organized Notch dynamics generate stereotyped sensory organ patterns in *Drosophila*. Science 356, 501, eaai7407

M. Trylinski, K. Mazouni and F. Schweisguth (2017). Intra-lineage fate decisions involve activation of Notch receptors basal to the midbody in *Drosophila* sensory organ precursor cells. Current Biology, 27, 1-9

Scientific or technical background required for work programs

All the expertise needed to carry out this project can be acquired during this internship. However, a strong interest in Cell Biology and Genetics/Development is needed.

Title of the work program 7

Deciphering the role of RNA modifications in the *Plasmodium falciparum* life cycle

Description of the work program

Keywords: epitranscriptomics; translation control; non-coding RNA; RNA-protein interactions; CRISPR/Cas9 genome editing

Malaria is the deadliest disease in human history. In 2015, nearly half a million died and more than 200 million people were infected with *Plasmodium falciparum* - the causative agent of the most severe form of malaria. Malaria pathogenesis results from the asexual reproduction inside erythrocytes where the parasite differentiates and replicates up to 32 daughter cells within 48 hours. The developmental stages are characterized by specific profiles of gene expression that are highly coordinated by an as-yet poorly-defined regulatory mechanism. Interestingly, transcription factors seem to play a small role in transcriptional regulation, and gene activation/repression is believed to be mainly achieved by reversible histone modifications and spatial nuclear organization. However, an emerging body of evidence demonstrates that post-transcriptional regulation (i.e. at the RNA level) through specific protein-RNA interactions significantly contributes to coordinated gene expression in the human host and might also play a role in other life cycle stages (i.e. in the mosquito vector). While these processes seem to be key to parasite virulence, many questions remain concerning the extent of protein-RNA interactions, the manner in which proteins recognize specific RNA transcripts, and the consequences of these interactions.

Most intriguingly, recent findings in model organisms point towards a high level of post-transcriptional regulation through chemical modifications on mRNA and non-coding RNA transcripts. We set out to characterize the so-called 'epitranscriptome' in *P. falciparum* with mass spectrometry and identified dozens of RNA modifications throughout the parasite life cycle. Of those, we found methylation of adenosine at N^6 (m^6A) to be the most abundant and highly dynamic RNA modification. Currently, we are attempting to characterize the m^6A methylation machinery, which includes knock down of the putative m^6A methyltransferase and genome-wide identification of individual m^6A sites.

Our current efforts and the main focus of the proposed project will be on characterizing the m^6A methylation-dependent processes and phenotypes in *P. falciparum*. Specific questions include:

1) Which proteins are involved in 'writing' m^6A methylation on RNA and which proteins specifically recognize this modification?
2) How do m^6A modifications affect mRNA translation and stability?
3) Does m^6A occur on types of RNA transcripts other than mRNA?
4) On a broader 'organismal' level, how does m^6A affect the parasite in its development and progression through the life cycle?

The Master student will learn and apply several targeted and genome-wide approaches to answer these questions. Protein immunoprecipitations will be used to identify new members of the m^6A methylation complex and RNA pull-downs will help to identify specific m^6A 'reading' proteins. Genome-wide approaches such as ribosome profiling will be used to measure translation efficiencies across the parasite life cycle in methylation-deficient versus wild-type parasites. In follow-up experiments, the student will have the opportunity to characterize individual proteins involved in RNA methylation by creating inducible knock out or knock down cell lines with CRISPR/Cas9. Importantly, the student will have the flexibility to investigate unforeseen and interesting developments in the proposed project.

The proposed project takes the emerging hot topic of post-transcriptional regulation and applies it to a pathogen that has a huge impact on global public health. The student will receive training in a wide variety of techniques in parasitology, cell and molecular biology, and genetics, ranging from parasite cell culture to generation and bioinformatic analysis of NGS data. To facilitate scientific interactions, BIHP has weekly lab meetings and participates in an annual lab and departmental retreat. Finally, each student in BIHP is encouraged to attend international workshops and conferences where she/he will have the opportunity to present her/his data and interact with experts from all over the world.

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

- Scherf, A., Lopez-Rubio, J. J., and Riviere, L. (2008) Antigenic variation in Plasmodium falciparum, *Annu Rev Microbiol 62*, 445-470.
- Bryant, J.M., Regnault, C., Scheidig-Benatar, C., Baumgarten, S., Guizetti, J., and Scherf, A. (2017) CRISPR/Cas9 genome editing reveals that the intron is not essential for *var2csa* gene activation or silencing in *Plasmodium falciparum*, *mBio*.
- Cubi, R., Vembar, S.S., Biton, A., Franetich, J.F., Bosson-Vanga, H., Moreno, A., Dereuddre-Bosquet, N., Le Grand, R., Scherf, A., and Mazier, D. (2017) Laser capture microdissection enables transcriptomic analysis of dividing and quiescent liver stages of *Plasmodium* relapsing species, *Cell Microbiol*.
- Guizetti, J., Barcons-Simon, A., and Scherf, A. (2016) *Trans*-acting GC-rich non-coding RNA at *var* expression site modulates gene counting in malaria parasite, *Nucleic Acids Res*.
- Zhang, Q., Siegel, T.N., Martins, R.M., Wang, F., Cao, J., Gao, Q., Cheng, X., Jiang, L., Hon, C.C., Scheidig-Benatar, C., Sakamoto, H., Turner, L., Jensen, A.T., Claes, A., Guizetti, J., Malmquist, N.A., and Scherf, A. (2014) Exonuclease-mediated degradation of nascent RNA silences genes linked to severe malaria, *Nature*.
- Ghorbal, M., Gorman, M., Macpherson, C. R., Martins, R. M., Scherf, A., and Lopez-Rubio, J. J. (2014) Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system, *Nat Biotechnol*.

Scientific or technical background required for work program

Basic experience in molecular and cell biology. Proficiency in English is a requirement

Title of the work program 8

Virulence gene expression in the blood and mosquito stages of *Plasmodium falciparum*

Description of the work program

Plasmodium falciparum is the most virulent species of malaria parasite, causing high rates of morbidity and mortality in those infected. *P. falciparum* erythrocyte infection and chronic infection depend on an immune evasion mechanism termed antigenic variation. Pathogenesis relies on monoallelic expression of *var* genes, which encode the PfEMP1 antigenic surface proteins that decorate infected erythrocytes and allow them to adhere to the microvasculature of the human host. Out of a family of ~60 *var* genes, only one is expressed at a given time while the rest are kept in a transcriptionally silent state. When immune pressure is mounted against one PfEMP1, the parasites switch to express a different member of the *var* gene family. Antigenic variation is used by several parasites to evade host immune systems, and understanding the transcriptional regulation of this process is critical for developing drugs to treat the resultant diseases.
The proposed project will investigate *var* gene transcription in the blood and mosquito stages of *P. falciparum*. The project will involve training in parasite cell culture and techniques in molecular biology such as cloning, parasite genome editing with CRISPR/Cas9, qPCR, chromatin immunoprecipitation, and next generation sequencing. The masters student will also be collaborating with the CEPIA platform at the Institut Pasteur, which performs parasite infections of *Anopheles* mosquitos. The data gathered during this project will have broad applications not only for the field of parasitology, but for the study of transcriptional regulation in other organisms and pathways.

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

- Scherf, A., Lopez-Rubio, J. J., and Riviere, L. (2008) Antigenic variation in Plasmodium falciparum, *Annu Rev Microbiol 62*, 445-470.
- Bryant, J.M., Regnault, C., Scheidig-Benatar, C., Baumgarten, S., Guizetti, J., and Scherf, A. (2017) CRISPR/Cas9 genome editing reveals that the intron is

not essential for *var2csa* gene activation or silencing in *Plasmodium falciparum*, *mBio*.
- Cubi, R., Vembar, S.S., Biton, A., Franetich, J.F., Bosson-Vanga, H., Moreno, A., Dereuddre-Bosquet, N., Le Grand, R., Scherf, A., and Mazier, D. (2017) Laser capture microdissection enables transcriptomic analysis of dividing and quiescent liver stages of *Plasmodium* relapsing species, *Cell Microbiol*.
- Guizetti, J., Barcons-Simon, A., and Scherf, A. (2016) *Trans*-acting GC-rich non-coding RNA at *var* expression site modulates gene counting in malaria parasite, *Nucleic Acids Res*.
- Zhang, Q., Siegel, T.N., Martins, R.M., Wang, F., Cao, J., Gao, Q., Cheng, X., Jiang, L., Hon, C.C., Scheidig-Benatar, C., Sakamoto, H., Turner, L., Jensen, A.T., Claes, A., Guizetti, J., Malmquist, N.A., and Scherf, A. (2014) Exonuclease-mediated degradation of nascent RNA silences genes linked to severe malaria, *Nature*.
- Ghorbal, M., Gorman, M., Macpherson, C. R., Martins, R. M., Scherf, A., and Lopez-Rubio, J. J. (2014) Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system, *Nat Biotechnol*.

Scientific or technical background required for work program

Basic experience in molecular and cell biology. Proficiency in English is a requirement.

Title of the work program 9

Role of nicotinic acetylcholine receptors in Alzheimer's disease in rodent models

Description of the work program

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by memory and cognitive deficits. The neuropathological hallmarks of this disease are amyloid-beta extracellular plaques and intracellular neurofibrillary tangles of tau protein in the brain, in association with a synaptic and neuronal dysfunction. AD patients display a loss of cholinergic neurons of the basal forebrain that project to both the neocortex and the hippocampus. They also show a considerable loss of high-affinity acetylcholine nicotinic receptors (nAChRs) containing the β2 subunit in the temporal cortex and the hippocampus. We have developed a novel animal model based on the expression of a mutated form the human amyloid precursor protein (APP) in the mouse hippocampus based of targeted injections of lentiviral vectors. This approach allowed us to express a human APP presenting three pathogenic mutations (Swedish, London and Austrian) in specific brain regions such as the dentate gyrus of the hippocampus. The expression of the mutated APP in the mouse hippocampus resulted in both the synthesis and accumulation of Abeta oligomers and in memory deficits. However, knockout mice lacking the β2 nicotinic subunit gene did not develop such memory deficits, which suggests a crucial role of β2 nAChRs in the deleterious effect of Abeta on memory function. We now aim at confirming and further understanding the role of β2 nAChRs in the memory impairment induced by viral transduction of mutant hAPP in the mouse hippocampus using adenovirus-associated virus (AAV) vectors, that provide a quicker and stronger expression of the transgene of interest, and by testing a novel β2*nAChR antagonist in our AD model.

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

Lombardo S, Catteau J, Besson M, Maskos U, A role for β2 nicotinic receptors in a model of local amyloid pathology induced in dentate gyrus, Neurobiol. Aging 2016 Jun;46:221-234.
*Koukouli F, Rooy M, Maskos U, Early and progressive deficit of neuronal activity patterns in a model of local amyloid pathology in mouse prefrontal cortex, 2016 Dec 20;8(12):3430-3449.
*Maskos U, Lombardo S, Catteau J, Besson M, Koukouli F. "*Inhibition of beta-2 nicotinic acetylcholine receptors to treat Alzheimer's disease pathology*". Patent application n° PCT/EP2017/061630.

Scientific or technical background required for work program

The student should have a good theoretical background in neuroscience and an interest in performing behavioural tasks with rodents. Previous experience in animal handling and immunohistofluorescence techniques would be very welcome.

Title of the work program 10

Analysis of the role of the secreted protein Lmo2595 in *Listeria monocytogenes* infection

Description of the work program

Listeria monocytogenes (*Lm*) is a foodborne human pathogen largely exploited as a model in infection biology. The pathogenicity of *Lm* is mediated by bacterial-encoded virulence factors, which promote entry and multiplication in host cells and escape from cellular immunity.
We have recently identified several *Lm*-specific proteins potentially involved in virulence.

This project aims to characterize one of these virulence factors named Lmo2595.

Our preliminary findings show that Lmo2595 is required for *Lm* virulence since deletion of the gene decreased *Lm* colonization in a murine model. Additionally, by raising specific antibodies against Lmo2595, we were able to show that the protein is expressed and secreted.

We now want to define the mechanism of action of Lmo2595 during infection at the molecular level. To do so, we will identify the binding partners of Lmo2595 in infected mammalian cells by immunoprecipitation coupled to mass spectrometry analysis.
The most promising hits will be tested for direct binding to Lmo2595 by in vitro pull-down assays with recombinant proteins. We will then assess the functional role of the interaction between Lmo2595 and its partners by looking at their stability, localization and biological activity.
The characterization of Lmo2595 function will provide novel findings on *Lm* virulence to expand our understanding of host-pathogen interactions, an indispensable requirement in the fight against pathogens of medical relevance.

Tutor/supervisor

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

1. Cossart, P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes.* Proc Natl Acad Sci USA. 2011; 108(49):19484-91

2. Cossart, P. and Lebreton, A. A trip in the “New Microbiology” with the bacterial pathogen *Listeria monocytogenes.* FEBS lett. 2014; 588(15):2437-45

https://www.youtube.com/watch?v=dlAPOa_QXAo

Scientific or technical background required for work program

The candidate will develop the project in the Unité des Interactions Bactéries-Cellules (Institut Pasteur), led by P. Cossart. The Cossart laboratory not only provides an excellent and stimulating scientific environment, but has also assembled an impressive number of tools to study *Lm* infection over the last 20 years.
The candidates are expected to have a strong interest in the field of cellular microbiology and/or microbiology. A background in mammalian cells manipulation, biochemistry and confocal microscopy will be appreciated but it is not strictly necessary.

Title of the work program 11

Functional analysis of developing neuronal precursors derived from human iPS Cells

Description of the work program

Schizophrenia is one of the top ten causes of long-term disability worldwide with a high economic cost associated. Like most psychiatric disorders, it is very genetically complex. A recent Genome Wide Association Study (GWAS) which identified 108 loci associated with schizophrenia found a strong link with the CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster on chromosome 15. Moreover, a recent study of the chromosomal interactions of those genes has shown that this cluster could have a regulatory role during development. We therefore want to delineate the role of genetic variation in nicotinic receptor genes for schizophrenia-relevant phenotypes. We hypothesise that these variations may contribute to early brain network formation and function, thereby shaping mental disease phenotypes.

One of the main obstacles for studying the molecular mechanisms underlying human neurodevelopment is the scarcity of experimental models. The discovery that neurons can be generated from human induced pluripotent stem cells (hiPSCs) paves the way for novel approaches that are stem cell-based. In a submitted paper (1), we developed a technique to follow the engraftment and integration of transplanted hiPSC-derived neuronal precursors in the cortex of mice. We are using post-mortem immunohistochemistry and *in vivo* two-photon imaging to quantify a progressive differentiation and maturation of the human neurons over eight months. We have already found that hiPSC derived neurons establish a mature dendritic pattern and axonal projections in the host brain. These data demonstrate that hiPSC-derived neuronal precursors can be efficiently transplanted, and follow an innate human developmental programme, in different cortical areas. This offers novel possibilities for the *in vivo* modeling and study of human cortical development, followed in real-time.

The development of the human brain is a challenging topic in neuroscience. The genetic heterogeneity of humans makes it even more complex to relate a given genome to developmental programs, and identify environmental factors interacting with the genetic profile of individuals. As a first step towards addressing these issues, several studies have demonstrated that human *embryonic* stem cells (hESCs) are able to mature and differentiate into functional neurons and glia, by using a mechanism similar to *in vivo* development (2, 3). Additionally, hESC transplantation has proved to be a very efficient and valuable tool to understand the mechanism of human cortical development (4, 5). Espuny-Camacho and colleagues transplanted hESC-derived cortical neurons into the newborn mouse brain and showed that they integrated robustly and established defined axonal projections and dendritic patterns corresponding to native cortical neurons. Over several months *in vivo* after transplantation, the human neurons showed a progressive maturation of their differentiation and connectivity (6). However, ethical issues have limited the use of hESCs (7, 8).

The drawbacks of using hESCs could be overcome by instead using human induced pluripotent stem cells (hiPSCs), being derived from human somatic cells, such as skin cells, by a combined action of pluripotent transcription factors (9, 10). Furthermore, the advent of hiPSCs makes it possible to study various psychiatric disorders, including autism spectrum disorders (ASDs) and schizophrenia (SCZ), which are heritable, complex genetic neurodevelopmental diseases (11).

We have been able show that hiPSC-derived neuronal precursors, after transplantation into the newborn mouse cortex, migrate from the graft and efficiently differentiate and mature over time. The morphological development of the graft was tracked

over several months post-injection, by post-mortem immunohistochemistry and confirmed by *in vivo* two-photon microscopy, thus offering the potential for *in vivo* modeling and study of human cortical neuron development, in real time.

We will now further follow the development of activity patterns in the human neurons using iPS cells stably transduced with a LV expressing the transgenic calcium indicator GCaMP6f, see Figure 1. The activity patterns will then be compared between iPS cells expressing the "wild-type" alpha5 subunit, and compared to the rs16969968 expressing line. Furthermore, the role of the CHRFAM7A human-specific gene will be compared

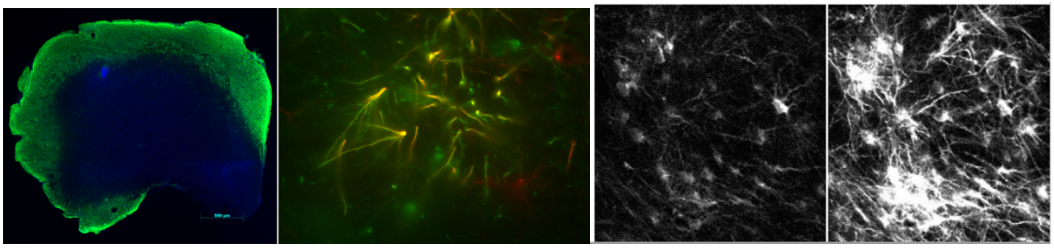

Figure 1. Generation of human-mouse chimaeric brains, and functional analysis
(Left) Extensive contribution of human neurons (GFP labelled, green) to mouse frontal cortex. Also non-neuronal cells are derived from the human iPS cells: astrocytes are labelled in red with a GFAP antibody, and yellow indicates co-label with GFP, identifying cells of human origin.
(Right) Transplanted human iPS-derived neurons transduced with a GCaMP6f lentiviral vector express strong fluorescence and exhibit spontaneous calcium transients.

During the internship, the student will participate in the histological and functional analysis of the injected cells. One part of the project will be to sample the brain of the injected mice at relevant time points and perform immunostainings, to characterise the human cells. Then, their morphology will be analysed using the tracing software Acapella.

For the functional part of the project, cranial windows will be opened on the adult mice, so they can be chronically observed under the 2-photon microscope. The mice will then be regularly imaged to compare the cell's activity throughout development.

1. D'Alessio R, et al. In vivo differentiation and long-term maturation of human iPSC-derived neuronal precursors followed over time. *submitted*.
2. Perrier AL, et al. (2004) Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc Natl Acad Sci U S A* 101(34):12543–8.
3. Hu B-Y, Du Z-W, Li X-J, Ayala M, Zhang S-C (2009) Human oligodendrocytes from embryonic stem cells: conserved SHH signaling networks and divergent FGF effects. *Development* 136(9):1443–52.
4. Gaillard A, Gaillard F, Roger M (1998) Neocortical grafting to newborn and adult rats: developmental, anatomical and functional aspects. *Adv Anat Embryol Cell Biol* 148:1–86.
5. Gaspard N, et al. (2008) An intrinsic mechanism of corticogenesis from embryonic stem cells. *Nature* 455(7211):351–7.
6. Espuny-Camacho I, et al. (2013) Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. *Neuron* 77(3):440–56.
7. Aboody K, Capela A, Niazi N, Stern JH, Temple S (2011) Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. *Neuron* 70(4):597–613.
8. Ramos-Zúñiga R, González-Pérez O, Macías-Ornelas A, Capilla-González V, Quiñones-Hinojosa A (2012) Ethical implications in the use of embryonic and adult neural stem cells. *Stem Cells Int* 2012:470949.
9. Park I-H, et al. (2008) Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451(7175):141–6.
10. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–76.
11. Brennand KJ, Gage FH (2011) Concise review: the promise of human induced pluripotent stem cell-based studies of schizophrenia. *Stem Cells* 29(12):1915–22.

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

Deflorio, C., Blanchard, S., Carisì, M. C., Bohl, D., & Maskos, U. (2017). Human polymorphisms in nicotinic receptors: a functional analysis in iPS-derived dopaminergic neurons. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *31*(2), 828–839. http://doi.org/10.1096/fj.201600932R

Koukouli, F., Rooy, M., Tziotis, D., Sailor, K. A., O'Neill, H. C., Levenga, J., Maskos, U. (2017). Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. *Nature Medicine*, *23*(3), 347–354. http://doi.org/10.1038/nm.4274

Scientific or technical background required for work program

Immunostaining, some animal handling experience, cell culture

Title of the work program 12

Analysis of the organization of Folate Receptor α and its exocytosis in epithelial and breast cancer cells

Description of the work program

Most human cancers derive from polarized epithelial cells that lost their polarity highlighting that loss of epithelial polarity is a critical step towards malignancy. Thus understanding the molecular mechanisms allowing the establishment and maintenance of epithelial polarity is of fundamental interest. Polarized epithelial cells possess an asymmetrical plasma membrane (PM) with apical and basolateral domains that differ drastically in their protein and lipid composition and in their functions. Epithelial polarity results from a selective sorting of proteins and lipids from the Golgi complex and their selective recycling to either apical or basolateral domain[1, 2]. Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are mostly apical PM proteins and are therefore a very good tool to study epithelial polarity. We showed that apical sorting of GPI-APs is governed by the capacity of GPI-APs to form high molecular weight complexes or clusters in the Golgi apparatus. Only GPI-APs organize in cluster in the Golgi are apically sorted while the monomeric form of the protein are targeted to the basolateral surface[3]. Importantly, clustering of GPI-APs in the Golgi regulates their apical sorting but also their apical plasma membrane organization and their biological activities. These results highlight the critical role of GPI-AP PM organization in the regulation of their biological activities and the strict correlation between the sorting mechanism (mediated by clustering in the Golgi) and PM organization. Recently we showed that cholesterol and calcium are masters regulators of Golgi GPI-APs organization[4-6](Lebreton et al. Submitted).

Folate receptor alpha (FRα) is an apical GPI-AP expressed in a restricted number of healthy cells (kidney, lung and breast). Importantly, FRα is overexpressed in several human cancers (breast, ovarian, lung, brain and colon) and nowadays several types of FR targeted therapies (antibodies or folic-acid drug) have been developed and are currently in clinical trials[7, 8]. While the precise role of FRα in human cancer is elusive, it is postulated that overexpression of FRα confers a growth advantage by increasing folate uptake (even under low folate conditions). In case of breast cancer (more than a million diagnoses each year) FRα expression is associated with hormone negative tumors and with triple negative breast cancers (TNBC) and overall with reduced disease survival[9, 10]. Furthermore, TNBCs are often associated with epithelial-mesenchymal transition (EMT), a cellular process by which epithelial cells transdifferentiate into mesenchymal cells[11].

Because of its involvement in human cancers understanding the regulation of sorting, trafficking, plasma membrane organization and biological function of FRα appear to be a critical issue both in cell and cancer biology.

The aim of this project is to analyze FRα Golgi organization and trafficking from the Golgi apparatus to the cell surface.

Golgi organization will be defined by biochemical approaches and the exocytosis from the Golgi to the apical cell surface will be determine by using the RUSH system that allows

synchronising protein exit from the Golgi complex and therefore to track in 3D in live the arrival of FRα to the apical surface (fast live spinning disk plus 3D image analysis)[12]. The research unit where the student will realize his project is highly dynamic, young and international.

Selected references

1 Apodaca, G., Gallo, L. I. and Bryant, D. M. (2012) Role of membrane traffic in the generation of epithelial cell asymmetry. Nat Cell Biol. **14**, 1235-1243

2 Ang, A. L., Taguchi, T., Francis, S., Folsch, H., Murrells, L. J., Pypaert, M., Warren, G. and Mellman, I. (2004) Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. J Cell Biol. **167**, 531-543

3 Paladino, S., Sarnataro, D., Pillich, R., Tivodar, S., Nitsch, L. and Zurzolo, C. (2004) Protein oligomerization modulates raft partitioning and apical sorting of GPI-anchored proteins. J Cell Biol. **167**, 699-709

4 Paladino, S., Lebreton, S., Tivodar, S., Formiggini, F., Ossato, G., Gratton, E., Tramier, M., Coppey-Moisan, M. and Zurzolo, C. (2014) Golgi sorting regulates organization and activity of GPI proteins at apical membranes. Nature chemical biology. **10**, 350-357

5 Paladino, S., Lebreton, S. and Zurzolo, C. (2015) Trafficking and Membrane Organization of GPI-Anchored Proteins in Health and Diseases. Curr Top Membr. **75**, 269-303

6 Lebreton, S., Paladino, S. and Zurzolo, C. (2008) Selective roles for cholesterol and actin in compartmentalization of different proteins in the Golgi and plasma membrane of polarized cells. J Biol Chem. **283**, 29545-29553

7 Assaraf, Y. G., Leamon, C. P. and Reddy, J. A. (2014) The folate receptor as a rational therapeutic target for personalized cancer treatment. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. **17**, 89-95

8 Gonen, N. and Assaraf, Y. G. (2012) Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy. **15**, 183-210

9 Necela, B. M., Crozier, J. A., Andorfer, C. A., Lewis-Tuffin, L., Kachergus, J. M., Geiger, X. J., Kalari, K. R., Serie, D. J., Sun, Z., Moreno-Aspitia, A., O'Shannessy, D. J., Maltzman, J. D., McCullough, A. E., Pockaj, B. A., Cunliffe, H. E., Ballman, K. V., Thompson, E. A. and Perez, E. A. (2015) Folate receptor-alpha (FOLR1) expression and function in triple negative tumors. PloS one. **10**, e0122209

10 O'Shannessy, D. J., Somers, E. B., Maltzman, J., Smale, R. and Fu, Y. S. (2012) Folate receptor alpha (FRA) expression in breast cancer: identification of a new molecular subtype and association with triple negative disease. Springerplus. **1**, 22

11 Kalluri, R. (2009) EMT: when epithelial cells decide to become mesenchymal-like cells. The Journal of clinical investigation. **119**, 1417-1419

12 Boncompain, G., Divoux, S., Gareil, N., de Forges, H., Lescure, A., Latreche, L., Mercanti, V., Jollivet, F., Raposo, G. and Perez, F. (2012) Synchronization of secretory protein traffic in populations of cells. Nat Methods. **9**, 493-498

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

1) Joint last author
Arkhipenko A, Syan S, Victoria GS, ***Lebreton S****, Zurzolo C*. PrPC undergoes basal to apical transcytosis in polarized epithelial MDCK cells.* ***PLoS One****. 2016 Jul 7;11(7)*

2) Joint First author
Paladino S*, **Lebreton S*** and Zurzolo C. GPI-APs sorting and plasma membrane organization in mammalian cells. ***Current topic in Membranes,*** *2015;75:269-303*

3) *Joint First author*
Paladino S*, **Lebreton S*,** Tivodar S, Formiggini F, Ossato G, Gratton E, Tramier M, Coppey-Moisan M and Zurzolo C. Golgi sorting regulates organization and activity of GPI proteins at apical membranes. ***Nature Chemical Biology*** *10, 350-357 (2014)*

4) *Joint First author*
Imjeti NS*, **Lebreton S*,** Paladino S, de la Fuente E, Gonzalez A, Zurzolo C
N-Glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells. *Molecular Biology of the Cell 2011 Dec;22(23):4621-34*

5) Schiff E, Campana V, Tivodar S, **Lebreton S**, Gousset K, Chiara Zurzolo. Co-expression of wild-type and mutant prion proteins alters their cellular localization and partitioning into detergent resistant membranes. ***Traffic*** *Jul;9(7):1101-15 (2008)*

6) **Lebreton, S.**, Paladino S., Zurzolo C. Selective roles for cholesterol and actin in compartmentalization ofdifferent proteins in Golgi and plasma membrane of polarized cells. ***Journal of Biology Chemistry*** *283(43) :29545-53 (2008)*

7) Paladino S, **Lebreton S**, Tivodar S, Campana V, Tempre R and Chiara Zurzolo
Different GPI-attachment signals modulate GPI-anchored proteins oligomerization and apical sorting. ***Journal of cell science*** *121(Pt121) :4001-7 (2008*

Scientific or technical background required for work program

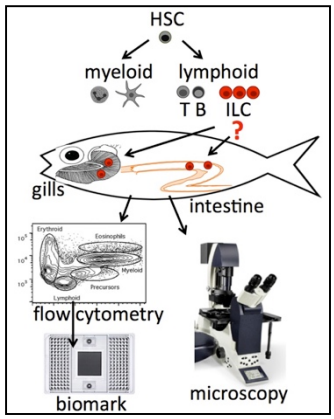
The realization of this research project will require cell culture, DNA transfection, biochemistry, confocal microscopy and fast live imaging using our spinning disk combined with image analysis and tracking.
The knowledge acquired will be on cell biology, protein trafficking organization in correlation with their function in normal epithelial and breast cancer cells

Title of the work program 13

Innate lymphoid cells and IL-22: functional analysis in zebrafish

Description of the work program

The immune system can be subdivided into two main arms: the innate and the adaptive. Recently, it was identified a new family of cells belonging to the innate system but that phenotypically and functionally resemble specific types of T lymphocytes, which belong to the adaptive system. In mice and in humans, these innate lymphoid cells (ILCs) play a critical role in host defense, in shaping the microbiota and in tissue repair at mucosal tissues via secreting cytokines such as IL-22. The innate system is more ancestral than the adaptive one, however, the existence and diversity of ILCs in organisms other than mammals remains unknown. This project will address the existence and role of the different subsets of ILCs and the conservation of IL-22 function in a distant relative, the zebrafish, a genetically tractable vertebrate with remarkable optical accessibility. Identification of ILCs in zebrafish will allow exploiting the advantages of this animal model to study early development and analyze ILC behavior at the whole animal level.



As described in the figure, this project will study the existence and diversity of ILCs as well as the function of IL-22 in zebrafish. To achieve this, we have generated mutant and reporter fish lines from which we will analyse their mucosae (gills and intestine) mainly via flow cytometry coupled to expression profiling and via microscopy of living fish and histological analysis.
The specific aims of the project for the M2 student will be any of the following depending on the interest of the student and on which months he/she will join the lab.

The main objectives of this project are:
1: Identification of innate lymphoid cells in zebrafish
a) Identification of blood cell lineages within mucosal sites of zebrafish. b) Analysis of lymphocyte-deficient zebrafish mutants. c) Characterization of zebrafish ILC diversity.
2: Study the function and cellular sources of zebrafish IL-22
a) Identification of cell types producing IL-22 and of stimuli inducing its expression. b) Identification of cell types responding to IL-22. c) Functional analysis of zebrafish IL-22

Among the techniques that will be used: Flow cytometry, real-time qPCR, microscopy in live fish and tissue sections, generation and use of transgenic fluorescent reporter fish as well as mutants via CRISPR/Cas9 system, gene expression profiling with different techniques.

Tutor/supervisor

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

Hernandez P. et al (2015). Interferon-λ and interleukin 22 act synergistically for the induction of interferon-stimulated genes and control of rotavirus infection. **Nature Immunology**. Jul;16(7):698-707

Murayama E. et al. (2015). NACA deficiency reveals the crucial role of somite-derived stromal cells in hematopoietic niche formation. **Nature Communications.** Sep 28;6:8375

Siupka P. et al (2014). The crystal structure of zebrafish IL-22 reveals an evolutionary, conserved structure highly similar to that of human IL-22. **Genes Immun**. Jul-Aug;15(5):293-302

Sarris M. et al. (2012). Inflammatory Chemokines Direct and Restrict Leukocyte Migration within Live Tissues as Glycan-Bound Gradients. **Current Biology** 22, 2375-82

Kissa K, Herbomel P (2010). Blood stem cells emerge from aortic endothelium by a novel type of cell transition. **Nature** 464, 112-5

Kissa K., et al. (2008). Live imaging of emerging hematopoietic stem cells and early thymus colonization. **Blood** 111, 1147-56

Scientific or technical background required for work program

Laboratory research experience in molecular biology and immunology courses attended during master programs. Previous experience doing real-time qPCR and knowledge in flow cytometry is a plus

Title of the work program 14

Roles of tunneling nanotubes in glioblastoma developement and resistance to therapies

Description of the work program

Glioblastoma (GBM) is the most common brain tumor in adults, with a very bad prognosis since the median survival is approximately 1 year with generally poor responses to all therapeutic modalities. Standard treatments associate surgery, radiotherapy and chemotherapy, but are often followed by recurrence of the tumor. Among the mechanisms of GBM cells adaptation or response to therapies those involving cell-to-cell communication are of great interest. In particular, the recent discovery of cellular connections called Tunneling nanotubes (connecting cancer cells and between cancer and stromal and/or endothelial cells) in several aggressive forms of cancer point appears to be key for cancer development and might be instrumental for its treatment (Osswald et al., 2015; Connor et al., 2015). Tunneling nanotubes (TNTs) are long and thin tubular structures between cells, allowing the direct transfer, from cytoplasm to cytoplasm, of a wide range of substances, including molecules, microRNAs, vesicles or organelles such as mitochondria (Weng, 2016) or lysosomes. These labile and transiently formed structures are still poorly understood; they contain actin filaments, sometimes microtubules, have a diameter from 100 nm to 800 nm and a length up to 100 µm (Abounit & Zurzolo 2012). TNTs have been involved in the transfer of aggregated proteins in neurodegenerative diseases (Parkinson, Alzheimer, Prions diseases). However, in cancers, their presence could be associated to bad prognosis and metastatic abilities of the tumors (Connor et al., 2015; Osswald et al., 2015).

The project consists in participating to the experiments performed on various GBM cell lines, aimed at understanding whether there is a link between the resistance of cells to therapies (chemotherapy or radiotherapy) and their ability to establish connections through TNTs. In particular the connections between GBM cells, treated or not, with surrounding cells (astrocytes or mesenchymal cells) will be assessed. We have already set up assays to quantify *in vivo* the number of TNTs using quantitative confocal microscopy (Gousset et al., 2013) and their functionality by measuring the transfer of fluorescently labeled vesicles from a donor cell population to acceptor cells (differently labeled) in co-cultures both by FACS and by quantitative microscopy (Abounit et al., 2015). We will apply these methods to various GBM/astrocytes cocultures and use similar assays to measure the transfer of mitochondria though TNTs using MitoTracker probes, as shown previously (Ahmad et al., 2014) in absence or after radio and/or chemotherapy.

This work will be done in the lab directed by Chiara Zurzolo at the Institut Pasteur.

Tutor/supervisor

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

Abounit, S., Bousset, L., Loria, F., Zhu, S., de Chaumont, F., Pieri, L., Olivo-Marin, J.-C., Melki, R., Zurzolo, C., 2016. Tunneling nanotubes spread fibrillar α-synuclein by intercellular trafficking of lysosomes. EMBO J 35, 2120–2138. doi:10.15252/embj.201593411

Abounit, S., Delage, E., Zurzolo, C., 2015. Identification and Characterization of Tunneling Nanotubes for Intercellular Trafficking. Curr Protoc Cell Biol 67, 12.10.1–12.10.21. doi:10.1002/0471143030.cb1210s67

Abounit, S., Zurzolo, C., 2012. Wiring through tunneling nanotubes--from electrical signals to organelle transfer. J Cell Sci 125, 1089–1098. doi:10.1242/jcs.083279

Chastagner, P., Rubinstein, E., Brou, C., 2017. Ligand-activated Notch undergoes DTX4-mediated ubiquitylation and bilateral endocytosis before ADAM10 processing. Sci Signal 10. doi:10.1126/scisignal.aag2989

Loria, F., Vargas, J.Y., Bousset, L., Syan, S., Salles, A., Melki, R., Zurzolo, C., 2017. α-Synuclein transfer between neurons and astrocytes indicates that astrocytes play a role in degradation rather than in spreading. Acta Neuropathol. doi:10.1007/s00401-017-1746-2

Victoria, G.S., Zurzolo, C., 2017. The spread of prion-like proteins by lysosomes and tunneling nanotubes: Implications for neurodegenerative diseases. J Cell Biol. doi:10.1083/jcb.201701047

Scientific or technical background required for work program

A solid background in cell biology would help.

Title of the work program 15

The role of ISG15 in the innate immune response of the female genital tract upon *Chlamydia trachomatis* infection

Description of the work program

Chlamydia trachomatis is an obligate intracellular bacterium targeting epithelial cells and a main causative agent for blindness and sexually transmitted infections. It undergoes a biphasic developmental cycle within a membrane-bound vacuole, in which it proliferates and refrains the immune response of host. Consequently, most of the patients with *C. trachomatis* infection are asymptomatic and untreated. This can lead to severe outcomes in the female genital tract, including infertility, pelvic inflammatory disease and ectopic pregnancy[1]. However, the mechanisms by which epithelial cells respond to *C. trachomatis* invasion, and how *C. trachomatis* counteracts these responses remain largely unclear. Interferon-stimulated gene 15 (ISG15), a ubiquitin-like molecule, acts either as a free molecule, or through conjugation to target proteins, a post-translational modification known as ISGylation[2,3]. So far studies on ISG15 activity have mostly focuses on its anti-viral property, with conflicting results (reviewed in [2]). Patients with inherited ISG15-deficiency show no increase in their susceptibility to viral infections, but are more susceptible to infection by mycobacteria[4]. Another study reveals that ISG15 interferes with the infection by *Listeria monocytogenes*[5]. We have observed that *C. trachomatis* infection induced ISG15 expression by human cervical epithelial Hela cells. The production of pro-inflammatory cytokines upon infection was increased in the absence of ISG15, suggesting the ISG15 expression refrains the innate immune response during *C. trachomatis* infection. The present project aims at investigating the mechanisms by which *C. trachomatis* induces ISG15 expression, and how ISG15 modulates *C. trachomatis*-induced inflammation. We will first confirm the *C. trachomatis*-induced ISG15 production in primary cervical epithelial cells isolated from patients undergoing hysterectomy. The involvment of signaling pathways in this ISG15 induction such as NF-κB and MAPK will be examined using pharmacological inhibitors and siRNA. Given that cyclic GMP-AMP synthase (cGAS) and cyclic di-AMP (c-di-AMP) mediate *C. trachomatis*-induced type I interferon expression[6,7], we will check the potential role of cGAS and c-di-AMP in *C. trachomatis*-induced ISG15 expression and implicated signals. To investigate the role ISG15 in modulating *C. trachomatis*-induced inflammation, recombinant human ISG15, stable cell line with overexpression of human ISG15 and ISG15 gene silence will be used. The potential mechanisms such as vacuole ubiquitination-mediated clearance and autophagy will be studied.
This project will reveal to what extent ISG15 expression modulates the innate immune response to *C. trachomatis* infection and may suggest new strategies to counteract the deleterious consequences of *C. trachomatis* infection.

References:

1. Elwell C, et al. Nat Rev Microbiol, 2016;14:385
2. Bogunovic D, et al. Exp Mol Med. 2013;45:e18
3. D'Cunha J, et al. Proc Natl Acad Sci USA, 1996;93:211
4. Bogunovic D, et al. Science, 2012;337:1684
5. Radoshevich L, et al. Elife, 2015;4:e06848
6. Zhang Y, J Immunol, 2014;193:2394
7. Barker JR, mBio, 2013;4:e00018

Tutor/supervisor

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

- Chu J, Zhang Q, Zhang T, Han E, Zhao P, Khan A, He C, Wu Y. Chlamydia psittaci infection increases mortality of avian influenza virus H9N2 by suppressing host immune response, Sci Rep 2016 Jul;6:29421
- 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, Elife 2016 Mar;5
- Pernet E, Guillemot L, Burgel PR, Martin C, Lambeau G, Sermet-Gaudelus I, Sands D, Leduc D, Morand PC, Jeammet L, Chignard M, Wu Y*, Touqui L*. Pseudomonas aeruginosa eradicates Staphylococcus aureus by manipulating the host immunity, Nat Commun 2014 Oct;5:5105 (*co-senior authors)

Scientific or technical background required for work program

The candidate should be motivated and a solid background in cell biology and microbiology is preferable.

Title of the work program 16

Role of mRNA methylation in bacteria-host-interactions

Description of the work program

Our lab is investigating different aspects of host pathogen interactions taking the bacterium *Listeria monocytogenes* as a model system. Since this bacterium is an enteropathogen, we are interested in the interplay between the bacterial pathogen and the intestinal microbiota during infection, and found that the *Listeria* has an impact on the composition of the microbiota (1). Recently, posttranscriptional modifications of mRNA were shown to play a role in the regulation of protein expression (2). Strikingly, we found in an ongoing study that the microbiota has an impact on methylation of mRNA in the host intestine. To further study the physiological role of this methylation, we are generating a conditional knock- out mouse model for the enzyme responsible for methylating mRNAs, METTL3.
The first part of the project includes the **analysis of tissue-specific knock- out mice** of METTL3 by **immunohistochemistry**, **western blotting** and **qRT PCR**.
In the second part of the project, we would like to study whether, similar to the gut flora, bacterial pathogens influence RNA methylation, and if this plays a role during infection.
The working program for this second part of the study includes **preparation of primary cells** from wild type and METTL3 knock- out mice, **infection with *Listeria monocytogenes*** and subsequent evaluation of the rate of infection and **analysis of mRNA methylation** by nucleoside mass spectrometry (in collaboration).
Techniques used for this project will cover the fields of cell biology, microbiology, and microscopy analysis. This project will benefit from the lab's longstanding expertise in host-bacteria interaction studies and from high-quality technologies and equipment available in the lab and in the Pasteur Institute.

(1) Quereda JJ, Dussurget O, Nahori MA, Ghozlane A, Volant S, Dillies MA, Regnault B, Kennedy S, Mondot S, Villoing B, Cossart P, Pizarro-Cerda J. Bacteriocin from epidemic Listeria strains alters the host intestinal microbiota to favor infection. Proc Natl Acad Sci U S A. 2016

(2) Zhao BS, Roundtree IA, He C. Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol.

Tutor/supervisor

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

Impens F, Rolhion N, Radoshevich L, Bécavin C, Duval M, Mellin J, García Del Portillo F,

Pucciarelli MG, Williams AH, Cossart P. N-terminomics identifies Prli42 as a membrane miniprotein conserved in Firmicutes Nat Microbiol. 2017

Quereda JJ, Dussurget O, Nahori MA, Ghozlane A, Volant S, Dillies MA, Regnault B, Kennedy S, Mondot S, Villoing B, Cossart P, Pizarro-Cerda J. Bacteriocin from epidemic Listeria strains alters the host intestinal microbiota to favor infection. Proc Natl Acad Sci U S A. 2016
Pereira JM, Hamon MA, Cossart P. A Lasting Impression: Epigenetic Memory of Bacterial Infections? Cell Host Microbe. 2016

Radoshevich L, Impens F, Ribet D, Quereda JJ, Nam Tham T, Nahori MA, Bierne H, Dussurget O, Pizarro-Cerdá J, Knobeloch KP, Cossart P. ISG15 counteracts Listeria monocytogenes infection. Elife. 2015

Eskandarian HA, Impens F, Nahori MA, Soubigou G, Coppée JY, Cossart P, Hamon MA. A role for SIRT2-dependent histone H3K18 deacetylation in bacterial infection. Science. 2013

Archambaud C, Nahori MA, Soubigou G, Bécavin C, Laval L, Lechat P, Smokvina T, Langella P, Lecuit M, Cossart P. Impact of lactobacilli on orally acquired listeriosis. Proc Natl Acad Sci U S A. 2012

Scientific or technical background required for work program

Background in biochemistry, biology, pharmacy or medicine. Some practical experience in cell biology, biochemistry or histology, e.g. cell culture, immunofluorescence microscopy, etc. would be useful.

Title of the work program 17

Comprehensive map of genetic interactions of GTPases regulating the intracellular trafficking using a CRISPR/Cas9 screening strategy

Description of the work program

Eukaryotic cells contain extensive internal membranes defining many compartments having each specific function: nucleus, mitochondria, endoplasmic reticulum (ER), the Golgi apparatus, lysosomes, endocytic and secretory vesicles. One of the major functions of this intricate vesicular-endomembrane network is to operate and control the sorting of proteins. From ER to Golgi, large families of small GTPases like Rabs or Arfs act as key regulators for all steps of the vesicular transport: vesicle formation, scission, targeting and fusion. Therefore, these families of proteins are essential for the homeostasis of eukaryotic cells. While a few numbers of these members have been extensively studied, little is known regarding the precise function of the vast majority of these regulators. A major difficulty stems from the redundancy of these proteins. The global objective of this project is to tackle this challenge and we propose to (i) better characterize the localization in living cells upon their endogenous expression, (ii) interrogate their function in cargo recycling and pathogen infection and (iii) elucidate their genetic interaction. To do so, we developed an approach based on CRISPR/Cas9 gene edition system to generate GFP Knock-In (KI) as well as Knock-Out (KO) within the human near-diploid HCT-116 cell line. A bank of plasmids for KO and KI has been already generated and an initial validation is underway. During this program, we propose to conduct a pilot assay focused on a limited set of genes (5 in total). First, we will Knock-In the Green Fluorescent Protein (GFP) sequence in frame with these five genes of interest as previously performed (Grassart et al, 2014). Successful KI cells will be directly selected at the single cell level by fluorescence activated cell sorting (FACS). Single cell clones will be verified by genotyping, sequencing, western blotting (WB) and fluorescence microscopy. Then, these cell lines will be used to generate Knock-Out at single cell and pool level. Briefly, KO will be evaluated by the loss of GFP fluorescence using FACS analysis. WB and gene sequencing will verify successful KO. Finally, all pair wise KO combination will be performed to analyze their genetic interaction on the maintenance of Golgi organization, cargo recycling and endocytosis. On the long-term perspective, the validation of this pilot assay will allow us to conduct a KO mid-scale experiment targeting the remaining complete family genes. Overall, this project will allow deciphering the regulation of intracellular compartmentalization.

Tutor/supervisor

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

1. Basquin C, Trichet M, Vihinen H, Malardé V, Lagache T, Ripoll L, Jokitalo E, Olivo-Marin JC, Gautreau A, **Sauvonnet** N. **2015**. Membrane protrusion powers clathrin-independent endocytosis of interleukin-2 receptor. **EMBO J**. 2015 Aug 13;34(16):2147-61
2. Lagache T, **Sauvonnet N**, Danglot L, Olivo-Marin JC. **2015**. Statistical analysis of molecule colocalization in bioimaging. **Cytometry A**. 2015 Jan 20.
3. Boucrot E, Ferreira A, Almeida-Souza A, Vallis Y, Howard G, Debard S, Bertot L, **Sauvonnet N** and McMahon HT. **2015** "Endophilin marks and controls a clathrin-independent endocytic pathway" **Nature**. 2015 Jan 22;517(7535):460-5.
4. **Grassart, A.,** Cheng, A. T., Hong, S. H., Zhang, F., Zenzer, N., Feng, Y., et al. (2014). Actin and dynamin2 dynamics and interplay during clathrin-mediated endocytosis. *The Journal of Cell Biology*, *205*(5), 721–735.
5. Mounier J, Boncompain G, Senerovic L, Lagache T, Chrétien F, Perez F, Kolbe M, Olivo-Marin JC, Sansonetti P, **Sauvonnet, N**. **2012.** Cholesterol relocation induced by the *Shigella* virulence factor IpaB inhibits host cell secretion by disrupting the Golgi complex and recycling network. **Cell Host and Microbe**, Sep 13;12(3):381-9.

Scientific or technical background required for work program

Cellular and molecular biology scientific and technical background.

Title of the work program 18

Design of TCR transfer vectors for HIV immunotherapy

Description of the work program

Rare cases of spontaneous control of HIV infection reveal that the human immune system has the capacity to mount an efficient antiviral response against HIV. Patients who contain HIV replication in the absence of therapy, called HIV Controllers, show signs of particularly efficient T cell responses, and maintain full CD4 helper function in the long term. We obtained recent evidence that CD4+ T cells of these rare patients preferentially express a particular set of shared T cell receptors (TCRs) directed at HIV capsid. These shared or "public" TCRs were found to detect Gag antigen with unusually high affinity. When transferred into healthy donor CD4+ T cells, the Gag-specific TCRs conferred properties characteristic of HIV Controller CD4+ T cell responses, including high antigen sensitivity and the capacity to produce multiple cytokines simultaneously. Of note, these TCRs were of sufficiently high affinity to also function in CD8+ T cells, in the absence of the CD4 coreceptor. Thus, both CD4+ and CD8+ T cells could be retargeted to the most conserved region of HIV-1 capsid by TCR transfer.

We propose to apply these findings to the field of HIV immunotherapy, by developing TCR transfer vectors that could be used to confer efficient antiviral responses against HIV. The main objective of this project will be to improve TCR lentivectors by inserting a fluorescent reporter gene and mutations that facilitate the pairing of the two vectorized TCR chains. This approach should optimize the expression level of the vectorized TCR, by avoiding competition from endogenous TCR chains. The lentivector constructs will be tested for their capacity to express the TCRs of interest by flow cytometry with TCRβ-specific antibodies and MHC II tetramers. The lentivectors will then be tested in primary T cells for their capacity to confer Gag-specific recognition, using intracellular cytokine staining. The cytotoxic function of TCR-transduced T cells will be analyzed by measuring the elimination of Gag-expressing target cells. The long-term goal will be to use these optimized TCR vectors in a humanized mouse model, to test their capacity at eliminating HIV-infected cells in vivo, and evaluate their potential as immunotherapeutic tools to restore efficient immune responses in HIV-infected patients.

Tutor/supervisor

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

- Vingert B., Perez-Patrigeon S., Jeannin P., Lambotte O., Boufassa F., Lemaître F., Kwok W.W., Theodorou I., Delfraissy J.F., Thèze J., and Chakrabarti L.A. ; for the ANRS EP36 HIV Controllers Study Group. (2010) HIV Controllers CD4+ T cells respond to minimal amounts of Gag antigen due to high TCR avidity. **PLoS Pathogens** 6(2): e1000780

- Chakrabarti L.A. and Simon V. (2010) Immune mechanisms of HIV control. **Current Opinion in Immunology** 22: 488-496.

- Vingert B., Benati D., Lambotte O., de Truchis P., Slama L., Jeannin P., Galperin M., Perez-Patrigeon S., Boufassa F., Kwok W. W., Lemaitre F., Delfraissy J. F., Theze J., and Chakrabarti L. A. (2012) HIV Controllers maintain a population of highly efficient Th1 effector cells in contrast to patients treated in the long term. **Journal of Virology** 86: 10661-10674.

- Granier C., Battivelli E., Lecuroux C., Venet A., Lambotte O., Schmitt-Boulanger M., Delaugerre C., Molina J. M., Chakrabarti L. A., Clavel F. and Hance, A. J. (2013) Pressure from TRIM5alpha contributes to control of HIV-1 replication by individuals expressing protective HLA-B alleles. **Journal of Virology** 87:10368-10380.

- Lissina A., Chakrabarti L.A., Takiguchi M., and Appay V. (2016) TCR clonotypes: molecular determinants of T cell efficacy against HIV. **Current Opinion in Virology** 16:77-85.

- Benati D., Galperin M., Lambotte O., Gras S., Lim A., Mukhopadhyay M., Nouël A., Campbell K.A., Lemercier B., Claireaux M., Hendou S., Lechat P., De Truchis P., Boufassa F., Rossjohn J., Delfraissy J.F., Arenzana-Seisdedos F., and Chakrabarti L.A. (2016) Public TCRs confer high-avidity CD4 responses to HIV Controllers. **Journal of Clinical Investigation** doi: 10.1172/JCI83792

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

Scientific or technical background required for work program

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

Title of the work program 19

Host immune response in severe pertussis

Description of the work program

Background: Despite vaccine implementation, pertussis still affects infants with a major morbidity and mortality burden worldwide. The most severe form, also called malignant or fulminant pertussis, is observed in some young infants and neonates. The mechanisms involved in pertussis severity are poorly understood. Our hypothesis is that it is due to a combination of microbiological factors and a specific inflammatory and immune response.

Objectives of the work program: To determine neonatal systemic immune response specificities to strains responsible for malignant pertussis.
Methodology:

An in vitro comparative study of neonatal immune response (cytokine and transcriptomic profiles) will be assessed after stimulation of newborn cord blood, with either strains from severe pertussis, or control strains. The transcriptional profiling will be assessed using the Human Immunology kit and NanoString nCounter®. Cytokines and chemokines secretion in the supernatant will be analyzed by Luminex.
The data of host immune responses will be then interpreted in the light of the complete genomic and epigenomic analysis of these isolates obtained in the laboratory, in order to identify microbial factors of *B. pertussis* associated with a specific host immune response.

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

- Toubiana J, Okada S, Hiller J, Oleastro M, Lagos Gomez M, Aldave Becerra JC, Ouachée-Chardin M, Fouyssac F, Girisha KM, Etzioni A, Van Montfrans J, Camcioglu Y, Kerns LA, Belohradsky B, Blanche S, Bousfiha A, Rodriguez-Gallego C, Meyts I, Kisand K, Reichenbach J, Renner ED, Rosenzweig S, Grimbacher B, van de Veerdonk FL, Traidl-Hoffmann C, Picard C, Marodi L, Morio T, Kobayashi M, Lilic D, Milner JD, Holland S, Casanova JL, Puel A. "Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype: an international survey of 274 patients from 167 kindreds." Blood. 2016 127(25): 3154-64

- Toubiana, J., Rossi, A. L., Grimaldi, D., Belaidouni, N., Chafey, P., Clary, G., Courtine, E., Pene, F. t al. "IMPDHII protein inhibits Toll-like receptor 2-mediated activation of NF-kappaB." J Biol Chem. 2016 286: 23319-33.
- Moura, A, A Criscuolo, H Pouseele, M. M. Maury, A. Leclercq, C. Tarr, J. T. Björkman, T. Dallman, A Reimer, V. Enouf, E. Larsonneur, H. Carleton, H. Bracq-Dieye, L. S. Katz, L. Jones, M. Touchon, M. Tourdjman, M. Walker, S. Stroika, T. Cantinelli, V. Chenal-Francisque, Z. Kucerova, E. P. C. Rocha, C. Nadon, K. Grant, E. M. Nielsen, B. Pot, P. Gerner-Smidt, M. Lecuit* and S. Brisse*. "Whole genome-based population biology and epidemiological surveillance of Listeria monocytogenes." Nat Microbiol. 2016 2:16185.
- Bialek-Davenet, S., A. Criscuolo, F. Ailloud, V. Passet, L. Jones, A. S. Delannoy-Vieillard, B. Garin, S. Le Hello, G. Arlet, M. H. Nicolas-Chanoine, D. Decre and S. Brisse Genomic Definition of Hypervirulent and Multidrug-Resistant Klebsiella pneumoniae Clonal Groups. Emerg Infect Dis. 2014 20, no. 11: 1812-20.
- Bouchez, V and Guiso N, Bordetella pertussis, Bordetella parapertussis, vaccines and cycles of whooping cough. Pathog Dis. 2015 73(7).
-Bouchez V, Hegerle N, Strati F, Njamkepo E, Guiso N. New data on Bordetella pertussis isolates no more producing one or two vaccine antigens. Vaccines (Basel). 2015 3(3):751-70.

Scientific or technical background required for work program

Scientific background in immunology.
Technical skills in molecular biology and cellular biology, would be preferred

Title of the work program 20

Investigation of cellular mechanisms leading to containment of an infectious agent (*Yersinia pseudotuberculosis*) upon arrival in the lymph node

Description of the work program

In this study, we will characterize the host's cellular components that are recruited and/or activated when *Y. pseudotuberculosis* reaches the lymph node. Mice will be infected with fluorescent bacteria in the footpad, and the popliteal lymph node will be taken at different times post infection for immunohistochemistry, flow cytometry, and confocal microscopy analyses. These analyses will give clues about the organization of the "granuloma" reaction and about the nature and proportion of the different cell populations. To determine which of these cells are critical for the formation of the cell response, specific cellular inhibitors and/or knock out mice (when available) will be used. Based on these results, intravital imaging of the *Y. pseudotuberculosis*-infected lymph node will be carried out in mice genetically modified to produce constitutively the fluorescent cell populations of interest, or in animals that received these fluorescently labeled cells prior to the infection. This study should provide an in depth understanding of the cellular mechanisms leading to the containment of an infectious agent upon arrival in the lymph node.

Tutor/supervisor

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

An encapsulated Yersinia pseudotuberculosis is a highly efficient vaccine against pneumonic plague. **Derbise A**, Cerdà Marín A, Ave P, Blisnick T, Huerre M, Carniel E, Demeure CE.PLoS Negl Trop Dis. 2012;6(2):e1528.
Oral vaccination against plague using Yersinia pseudotuberculosis. Demeure CE, **Derbise A**, Carniel E. Chem Biol Interact. 2017 Apr 1;267:89-95.

Scientific or technical background required for work program

No specific background is required.

Title of the work program 21

Actin-activated bacterial nucleotidyl cyclase toxins

Description of the work program

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

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

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

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

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

References:

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

Keywords:

bacterial toxins, nucleotidyl cyclase, cNMP, actin, ExoY

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

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

Scientific or technical background required for work program

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

Title of the work program 22

Synthetic investigation on bacterial oligosaccharides of interest for vaccine development

Description of the work program

Bacterial surface polysaccharides are important virulence factors as well as major targets of the host humoral immune response induced following natural infection. This observation has led to the development of polysaccharide-protein conjugate vaccines, a major breakthrough in the field of infectious diseases. Yet, there is no vaccine for shigellosis or bacillary dysentery, one of the top four diarrheal diseases circulating in the pediatric population. Disease is caused by Gram negative enteroinvasive bacteria named *Shigella*.

Instead of working with *Shigella* polysaccharides extracted from cell culture, we have addressed the development of a *Shigella* vaccine by use of synthetic oligosaccharides designed to act as functional mimics of the natural polysaccharide antigens. A first vaccine candidate was developed against *Shigella flexneri* 2a, the most prevalent serotype. It encompasses a synthetic pentadecasaccharide corresponding to an O-antigen segment made of three basic repeating units.

The proposed project is meant to broaden the strategy to another prevalent *Shigella* serotype. The successful candidate will be in charge of developing efficient syntheses of panels of type-specific polysaccharide fragments equipped with a linker for site-selective conjugation. Multiple-step chemical strategies based on optimized protecting group pattern and glycosylation chemistry shall enable the synthesis of oligosaccharides of increasing length and complexity. Developments will go beyond state-of-the-art glycochemistry to tackle some remaining challenging issues such as regio- and stereocontrol during glycosylation, non-stoichiometric substitutions, orthogonal protecting group manipulation, and/or robustness validation on large scale for compatibility with technical transfer. Synthetic targets and intermediates will be purified by flash chromatography and crystallization. The purified compounds will be characterized by NMR and MS.

Tutor/supervisor

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

Chassagne, P.; Fontana, C.; Guerreiro, C.; Gauthier, C.; Phalipon, A.; Widmalm, G.; Mulard, L. A., Structural studies of the *O*-acetyl containing O-antigen from a *Shigella flexneri* serotype 6 strain and synthesis of oligosaccharide fragments thereof. *Eur J Org Chem* **2013**, 4085-106.

Pfister, H.; Mulard, L. A., Synthesis of the zwitterionic repeating unit of the O-antigen from *Shigella sonnei* and chain elongation at both ends. *Org Lett* **2014**, 16, 4892-5.

Salamone, S.; Guerreiro, C.; Cambon, E.; André, I.; Remaud-Siméon, M.; Mulard, L. A., Programmed chemo-enzymatic synthesis of the oligosaccharide component of a synthetic carbohydrate-based antibacterial vaccine candidate. *Chem Commun* **2015**, 51, 2581-4. *Featured article*

Boutet, J.; Blasco, P.; Guerreiro, C.; Thouron, F.; Dartevelle, S.; Nato, F.; Cañada, F. J.; Ardá, A.; Phalipon, A.; Jiménez-Barbero, J.; Mulard, L. A., Detailed investigation of the immunodominant role of O-antigen stoichiometric O-acetylation as revealed by synthesis, immunochemistry, and NMR studies for *Shigella flexneri* 3a. *Chem Eur J* **2016**, 22, 10892-911. *Hot paper*

Hu, Z.; Bongat White, A. F.; Mulard, L. A. Efficient iterative synthesis of O-acetylated tri- to pentadecasaccharides related to the lipopolysaccharide of *Shigella flexneri* type 3a *via* di- and trisaccharide glycosyl donors. *Chem Asian J* **2017**, 12, 419-39.

Scientific or technical background required for work program

Strong interests and skills in the theory and experimental aspects of organic synthesis, including some background in the field of multi-step synthesis, purification (chromatography …) and analysis (NMR in particular) of complex molecules, are desirable. Prior experience in oligosaccharide synthesis added to interest in methodology, reaction optimization and scale up validation, would be an asset.
Applicants are expected to be highly motivated and curious. They should enjoy teamwork and show good communication skills. Good knowledge of English is recommended. Some knowledge of French would be an advantage.
A six-month fellowship or more is preferred.

Title of the work program 23

Phenotypic and functional analysis of genome instability during *Leishmania donovani* evolutionary adaptation

Description of the work program

Scientific background:

Leishmaniases are human vector-borne parasitic diseases causing severe morbidity and mortality in 98 countries throughout the world. Failing chemotherapy, inappropriate diagnosis and lack of vaccines are major obstacles for the eradication of leishmaniasis. Key to the pathogenicity of *Leishmania* is the remarkable capacity of these parasites to adapt to their host environments by stage differentiation of extracellular promastigotes within the sand fly vector, and intracellular amastigotes in mammalian host cells. Using HTSeq and phenotypic analyses we recently showed that aside adaptive differentiation, *Leishmania* exploits genome instability for environmental adaptation. Genome analysis of hamster-derived parasites revealed that fitness gain during adaptation to *in vitro* culture correlates to selection of stable trisomies for specific chromosomes, and the deletion of a NIMA-related protein kinase. HTseq analysis of clones derived from a population of culture adapted parasites further confirmed these results and indicated positive and purifying selection occurring on individual clones that lead to loss of heterozygosity. Given that culture adaptation causes virulence attenuation, we speculate that the expression of the deleted protein kinase and the maintenance of a heterozygous genome are essential for *Leishmania* infectivity. The project will test these hypotheses performing a detailed phenotype analysis of individual clones and kinase-transgenic/null mutant parasites.

Project:

Based on our previous results the project will follow two main objectives: (1) Phenotypic characterization of the clones derived from culture-adapted parasites and correlation of phenotypic differences to ploidy, heterozygosity or changes in gene copy number; (2) Investigate the role of the identified NIMA-like kinase in parasite growth using gain-of-function (transgenic expression) or loss of function (CRISPR/Cas9-mediated gene knock out, KO) approaches.

Experimental plan:

Experiments	Time (months)
Cloning of the kinase gene into pLEXSY (PCR from genomic DNA, construction of the plasmids) for overexpression Preparation of pTplasmid and primers for KO	1-2
Phenotypic characterization of clones (growth curves, infectivity, genome stability)	1-2
Selection and validation of transgenic and KO parasites	3
Phenotypic characterization of transgenic and KO parasites (growth curves, microscopy, infectivity…)	4-6

Tutor/supervisor

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

- *Haplotype selection as an adaptive mechanism in the protozoan pathogen Leishmania donovani.* P. Pieto Barja et al. Nature EcolEvol 2017, *in press*.
- *Modulation of Aneuploidy in Leishmania donovani during Adaptation to Different In Vitro and In Vivo Environments and Its Impact on Gene Expression.* Dumetz et al. MBio 2017.
- *Phenotypic Characterization of a Leishmania donovani Cyclophilin 40 Null Mutant.* Yau WL et al. J Eukaryot Microbiol 2017.
- *Probing druggability and biological function of essential proteins in Leishmania combining facilitated null mutant and plasmid shuffle analyses.* Dacher M et al. Mol Microbiol 2014.
- *Quantitative proteome profiling informs on phenotypic traits that adapt **Leishmania** donovani for axenic and intracellular proliferation.* Pescher P et al. Cell Microbiol 2011

Scientific or technical background required for work program

Basic knowledge in parasitology or microbiology.
Skills in basic molecular biology techniques (PCR, cloning...), FACS and cell culture would be welcome but the student will be trained in the lab in all the techniques required for the project as well as the good practises to work in BSL2+ conditions to be used for *Leishmania donovani* parasites.

Title of the research project 24

Human infection with zoonotic simian foamy retroviruses: role of virological and immunological factors in restricting viral emergence

Description of the research project

The public health relevance of **retroviruses of zoonotic origin** is illustrated by the human immunodeficiency virus (HIV) epidemic, the worldwide distribution of human T-lymphotropic viruses (HTLVs), and the morbidity and mortality associated with both sets of viruses. Both HIVs and HTLVs emerged in the human population after several cross-species transmission events involving retroviruses (SIVs and STLVs) endemic in non-human primates (NHPs). Moreover, a third genus of complex retroviruses, the foamy viruses, some of which are of simian origin, can also establish persistent infections in humans. **Simian foamy viruses (SFVs)** are widespread and highly prevalent in many NHP species. These viruses can be readily isolated from the saliva and oropharyngeal secretions of infected NHPs. **Penetrating bite wounds** therefore constitute a potential route of transmission to humans, leading to the establishment of **life-long persistent infection**. **Human infection with zoonotic SFVs constitutes a unique, natural model for studies of the restriction of retrovirus emergence in humans**.
Having shown that neutralizing antibodies are present at high titers in most SFV-infected individuals and that they target a highly conserved region of the viral envelope, **we now aim to define the antigenic epitopes**. A three-step strategy is planned: *in silico* prediction of antigenic peptides, neutralization assay based on competition with linear peptide or protein subdomains, functional validation of virus vulnerability sites using viral vectors. The fellow will have the opportunity to perform antigenic peptide prediction with bioinformatics tools, neutralization assays with replicating viruses and viral vectors, as well as ELISA and flow-cytometry assays.

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Selected publications or patents of the Research Group offering this research project

Avettand-Fenoel V, Blanche S, Le Chenadec J, Scott-Algara D, Dollfus C, Viard JP, Bouallag N, Benmebarek Y, Riviere Y, Warszawski J, Rouzioux C, Buseyne F. Relationships between HIV disease history and blood HIV-1 DNA load in perinatally infected adolescents and young adults: the ANRS-EP38-IMMIP study. **J Infect Dis**. 2012;205:1520-8. doi: 10.1093/infdis/jis233. PubMed PMID: 22427678.

Blanche S, Scott-Algara D, Le Chenadec J, Didier C, Montange T, Avettand-Fenoel V, Rouzioux C, Melard A, Viard JP, Dollfus C, Bouallag N, Warszawski J, Buseyne F. Naive T lymphocytes and recent thymic emigrants are associated with HIV-1 disease history in french adolescents and young adults infected in the perinatal period: the ANRS-EP38-IMMIP study. **Clin Infect Dis**. 2014;58:573-87. doi: 10.1093/cid/cit729. PubMed PMID: 24253249.

Rua R, Betsem E, Montange T, Buseyne F, Gessain A. In vivo cellular tropism of gorilla simian foamy virus in blood of infected humans. **J Virol**. 2014;88:13429-35. doi: 10.1128/jvi.01801-14. PubMed PMID: 25210185; PMCID: PMC4249094.

Richard L, Rua R, Betsem E, Mouinga-Ondeme A, Kazanji M, Leroy E, Njouom R, Buseyne F, Afonso PV, Gessain A. Cocirculation of Two env Molecular Variants, of Possible Recombinant Origin, in Gorilla and Chimpanzee Simian Foamy Virus Strains from Central Africa. **J Virol**. 2015;89:12480-91. doi: 10.1128/jvi.01798-15. PubMed PMID: 26446599; PMCID: PMC4665256.

Lambert C, Rua R, Gessain A, Buseyne F. A new sensitive indicator cell line reveals cross-transactivation of the viral LTR by gorilla and chimpanzee simian foamy viruses. **Virology**. 2016;496:219-26. doi: 10.1016/j.virol.2016.06.010. PubMed PMID: 27348053.

Buseyne F, Gessain A, Soares MA, Santos AF, Materniak-Kornas M, Lesage P, Zamborlini A, Lochelt M, Qiao W, Lindemann D, Wohrl BM, Stoye JP, Taylor IA, Khan AS. Eleventh International Foamy Virus Conference-Meeting Report. **Viruses**. 2016;8. doi: 10.3390/v8110318. PubMed PMID: 27886074; PMCID: PMC5127032.

Scientific or technical background required for the research project

Scientific background: Virology and/or immunology.
Technical background: Cell culture, viral titration, ELISA

Title of the work program: 25

Role of the viral non-structural NS1 lipoprotein in Dengue pathogenesis

Description of the work program

Dengue virus (DENV) causes a flu-like disease (dengue fever, DF) in humans that can progress to hemorrhagic fever (DHF) and a life-threatening shock syndrome (DSS). The four serotypes of DENV are spread around the globe in tropical areas, threatening about 75% of the global population. Epidemics are associated with factors such as rainfalls, high population density and trading. More than 100 million cases of DF are estimated to occur every year, leading to over half a million of severe DHF and several tens of thousand deaths. There is currently no vaccine or specific treatment commercially available.

This work program aims to study the role of the viral nonstructural protein 1 (NS1) in dengue pathogenesis. Our laboratory and others have previously found that the NS1 protein of flaviviruses circulates in the bloodstream of DENV-infected patients, at levels ranging on average from 100 ng to 1 µg/mL (Alcon *et al.,* 2002). The team further showed that NS1 is secreted as a hexameric lipid carrier that shares similarities with endogenous high and low density lipoproteins (HDL and LDL) (Gutsche *et al.*, 2011). Moreover, that hexameric soluble viral particle is able to interact with cell surfaces, enter through endocytosis and potentiate further infection. The liver is a major site of the lipoprotein metabolic pathway, with a predominant role in sensing, recycling and producing lipids and lipoproteins to ensure vascular homeostasis or promote inflammation. It also appears to be a major target for dengue virus infection and NS1 interaction (Alcon *et al*., 2002). Macrophages in the bloodstream are responsible for the uptake and recycling of cholesterol by HDLs, promoting the efflux from the endothelium wall to the liver. This cell type also happens to be a major target for dengue virus infection (Chaturvedi *et al.,* 1983).

We are currently analyzing the mode of interaction of the NS1 protein with hepatic cells and macrophages, identifying the binding modalities and the signals that may be triggered upon binding to the cell surface or during internalization. During this training period, the Erasmus student will learn how to characterize and produce the NS1 lipoprotein and mutants already designed by the team, displaying different associations with lipids. The student will assess the binding of NS1 viral protein to lipid-rich structures by biochemical and biophysical methods. The student will also study the interaction of the protein with target cells by immunofluorescence and assess their potential pro-inflammatory activity by ELISA.

Overall, these experiments will provide information on the mechanisms by which NS1 triggers deleterious signals, induces cellular dysfunctions and impacts disease progression in the human host.

The work program includes various types of experiments and tasks the student will be part of, including :

- **Protein production**
- **Bio-Layer Interferometry**
- **Cell culture**
- **Epifluorescent microscopy**
- **Fluorescent-activated cell sorting (FACS)**
- **Enzyme-linked immunosorbent assay (ELISA)**

In general terms, the Erasmus student will assist and collaborate with the team of researchers in the design of experiments. He/She will contribute to the experimental work and learn how to format results for discussions and publications.

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

Mounce BC, Cesaro T, Moratorio G, Hooikaas PJ, Yakovleva A, Werneke SW, Smith EC, Poirier EZ, Simon-Loriere E, Prot M, Tamietti C, Vitry S, Volle R, Khou C, Frenkiel MP, Sakuntabhai A, Delpeyroux F, Pardigon N, Flamand M, Barba-Spaeth G, Lafon M, Denison MR, Albert ML, Vignuzzi M. Inhibition of polyamine biosynthesis is a broad-spectrum strategy against RNA viruses. J Virol. 2016 Aug 17.

Léger P, Tetard M, Youness B, Cordes N, Rouxel RN, Flamand M, Lozach PY. Differential Use of the C-Type Lectins L-SIGN and DC-SIGN for Phlebovirus Endocytosis. Traffic. 2016 Jun;17(6):639-56.

Flamand M. The flavivirus NS1 protein's mysteries unveiled? Bioessays. 2015 May;37(5):472.

Fragnoud R, Flamand M, Reynier F, Buchy P, Duong V, Pachot A, Paranhos-Baccala G, Bedin F. Differential proteomic analysis of virus-enriched fractions obtained from plasma pools of patients with dengue fever or severe dengue. BMC Infect Dis. 2015 Nov 14;15:518.

Andries AC, Duong V, Ly S, Cappelle J, Kim KS, Lorn Try P, Ros S, Ong S, Huy R, Horwood P, Flamand M, Sakuntabhai A, Tarantola A, Buchy P. Value of Routine Dengue Diagnostic Tests in Urine and Saliva Specimens. PLoS Negl Trop Dis. 2015 Sep 25;9(9):e0004100.

Gutsche I[1], Coulibaly F, Voss JE, Salmon J, d'Alayer J, Ermonval M, Larquet E, Charneau P, Krey T, Mégret F, Guittet E, Rey FA, Flamand M. Secreted dengue virus nonstructural protein NS1 is an atypical barrel-shaped high-density lipoprotein. Proc Natl Acad Sci U S A. 2011 May 10;108(19):8003-8.

Alcon-LePoder S[1], Drouet MT, Roux P, Frenkiel MP, Arborio M, Durand-Schneider AM, Maurice M, Le Blanc I, Gruenberg J, Flamand M. The secreted form of dengue virus nonstructural protein NS1 is endocytosed by hepatocytes and accumulates in late endosomes: implications for viral infectivity. J Virol. 2005 Sep;79(17):11403-11.

Alcon S[1], Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J Clin Microbiol. 2002 Feb;40(2):376-81.

Scientific or technical background required for work programs

A good background in biology will be necessary to participate to the work program. In addition, some knowledge in biochemistry or cell biology and previous laboratory experience will facilitate immersion into the project and will therefore be very much appreciated.
Curiosity, enthusiasm and commitment are essential for the training to be successful and rewarding for everyone

Title of the work program 26:

Control of innate and adaptive immune responses by genetic variants associated with chronic inflammatory diseases

Description of the work program

Context: The past years have been marked by a leap forward in our insight of the genetic basis of many diseases. In particular, genome-wide association studies (GWAS) have provided detailed information about the genetic variants associated with chronic inflammatory diseases. These studies have highlighted many genes linked to signalling pathways that were not known to be involved in pathogenesis, and have pointed to new directions in the study of disease mechanisms. A subset of these genes are common to several diseases, suggesting that these conditions may share pathogenic pathways. GWAS also provided conclusive evidence for a key role of the immune system in the pathogenesis of these diseases, because many of the identified loci map to genes involved in different immune processes. However, for most SNPs, the mechanism by which they affect pathogenesis and the cell populations in which they act are still unknown.
The challenge of the post-GWAS era is to understand how these variants affect pathogenesis, to allow translation of genetic data into better diagnostics and innovative treatment strategies. To meet this challenge, it is necessary to design studies that enable the simultaneous analysis of cellular signalling pathways and genetic networks in patient samples, in order to establish a link between genotype, cellular phenotype/function, and pathology. This approach will improve our fundamental knowledge of the pathogenic mechanisms, and will help identify novel and relevant diagnostic tools, biomarkers and therapeutic targets.

General objectives: This project explores how genetic variants shape innate and adaptive immune cell functions via transcriptional and epigenetic mechanisms, using spondyloarthritis (SpA) as a model. The general goal is to improve our understanding of the molecular basis of SpA pathogenesis and of the immune cell populations involved. As a translational research goal, we aim at identifying immunologic signatures that can predict responsiveness to TNF blockers in SpA patients.

Ongoing research:
Define how genetic variants affect gene expression in whole-blood cultures and in specific immune cell populations that have been associated with SpA pathology
Previous work from our lab has shown that the impact of disease-associated SNPs on immune cell function can be measured when the immune system is challenged, rather than at steady state (Coffre et al, 2013). We will therefore analyze the effects of SNPs associated with inflammatory disease on the immune response in SpA patients in whole blood stimulation cultures (“TruCulture” assays, Duffy et al, 2014) and in purified immune cell populations that have been implicated in the pathogenesis of SpA. Isolated cell populations will be stimulated for different times prior to gene expression analysis. We will correlate

DNA variations with gene expression and cytokine/chemokine secretion in the stimulated cultures by expression quantitative trait loci (eQTL) mapping.

Specific question to be addressed by the student:
Guided by the lab head and in collaboration with a PhD student, the student will analyze gene expression in the samples we have collected. This will involve extraction of RNA, gene expression analysis using nCounter technology (Nanostring) and data analysis (comparison between groups, correlation with protein expression data, eQTL mapping).

Tutor/supervisor

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Profile on http://www.researchgate.net/ (if applicable):	Web site address of the lab: http://www.pasteur.fr/en/research/immunology/units-groups/immunoregulation

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

1. Lim AI, Menegatti S, Bustamante J, Le Bourhis L, Allez M, Rogge L, Casanova JL, Yssel H, Di Santo JP. 2016. IL-12 drives functional plasticity of human group 2 innate lymphoid cells. *J Exp Med* 213: 569-83
2. Hasan M, Beitz B, Rouilly V, Libri V, Urrutia A, Duffy D, Di Santo JP, Mottez E, Quintana-Murci L, Albert ML, Rogge L, Milieu Interieur Consortium. 2015. Semi-automated and standardized cytometric procedures for multi-panel and multi-parametric whole blood immunophenotyping. *Clin Immunol* 157: 261-76
3. Duffy D, Rouilly V, Libri V, Hasan M, Beitz B, David M, Urrutia A, Bisiaux A, Labrie ST, Dubois A, Boneca IG, Delval C, Thomas S, Rogge L, Schmolz M, Quintana-Murci L, Albert ML, Milieu Interieur C. 2014. Functional Analysis via Standardized Whole-Blood Stimulation Systems Defines the Boundaries of a Healthy Immune Response to Complex Stimuli. *Immunity* 40: 436-50
4. Dong S, Maiella S, Xhaard A, Pang Y, Wenandy L, Larghero J, Becavin C, Benecke A, Bianchi E, Socie G, Rogge L. 2013. Multiparameter single-cell profiling of human CD4+FOXP3+ regulatory T-cell populations in homeostatic conditions and during graft-versus-host disease. *Blood* 122: 1802-12
5. Coffre M, Roumier M, Rybczynska M, Sechet E, Law HK, Gossec L, Dougados M, Bianchi E, Rogge L. 2013. Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. *Arthritis Rheum* 65: 1510-21

Scientific or technical background required for work programs

A good knowledge of basic principles of immunology and genetics is important to understand the biologic question addressed.