Projects Fall 2016

RESEARCH CENTRE

Legal name: **Institut Pasteur**

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

Country: **France**

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Brief description of your Institution

Please consider that this description can be used in publications about the project and published on the web site www.unipharmagraduates.it

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 2800 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:

Maximum of 30.

The laboratories at Pasteur have proposed 28 projects for Erasmus internships (see following pages). The same projects will also be sent to other partner Universities in additional countries. Unipharma-Graduates will be informed as soon as a project is no longer available for selection by a Unipharma fellow.

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:

Optimization of a CRISPR/Cas9 system in Cryptococcus neoformans

Description of the work program

Cryptococcus neoformans is a yeast responsible for more than 600 000 deaths per year in the world (1). Although a large number of molecular tools are now available to study its biology and genetics, efficient directed point mutation remains a challenge. The objective of the program is to create and validate a CRISPR Cas9 system to be used in this pathogenic basiomycete yeast. This system is nowadays very commonly used to modify the genomes a large number of eukaryotic organisms including fungi (2). During its internship, the selected student will be in charge of the construction of different plasmids and in the optimization of the protocols needed to achieve efficiently a point mutation in the C. neoformans genome. Microbiology and molecular biology tools will be used. Of course the student will used the technical and theoretical expertise from the unit.

(1) Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, Bahn YS. 2015. Cryptococcus neoformans and Cryptococcus gattii, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med 4:a019760.
(2) Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH. 2015. A CRISPR-Cas9 System for Genetic Engineering of Filamentous Fungi. PloS One 10:e0133085

Tutor/supervisor

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

Goebels C., Thonn A., Gonsalez-Hilarion S., Rolland O., Moyrand F., Beilharz T. H. & **Janbon, G**. (2013) Introns regulate gene expression in *Cryptococcus neoformans* in a Pab2p dependent pathway. *PLoS Genetics* **9,** e1003686. (Recommended by F1000).

Janbon G., et al (2014) Analysis of the genome and transcriptome of *Cryptococcus neoformans* var. *grubii* reveals complex RNA expression and microevolution leading to virulence attenuation *PLoS Genetics* **10,** e1004261. (Recommended by F1000)

Wollschlaeger C., Trevijano-Contador N., Wang X., Legrand M., Zaragoza O., Heitman J., & **Janbon, G.** (2014) Distinct and redundant roles of exonucleases in *Cryptococcus neoformans*: Implications for virulence and mating. *Fungal Gen. Biol*. **73,** 20-28.

Kim H., Jung K.W., Maeng S., Chen Y.C., Shin J., Shim J.E., Hwang S., **Janbon G**., Kim T., Heitman J., Bahn Y.S., & Lee I. (2015) Network-assisted genetic dissection of pathogenicity and drug resistance in the opportunistic human pathogenic fungus *Cryptococcus neoformans. Sci. Rep.* **5,** 8767. (Recommended by F1000)

Jung K.W., … **Janbon G**., … Lee Y.W. & Bahn Y.S. (2015) Systematic functional profiling of transcription factor networks in *Cryptococcus neoformans. Nature Com.* **7,** 6757 ((Recommended by F1000).

Gonzalez-Hilarion S., Paulet D., Lee K.T., Hon C.C, Mogensen E., Moyrand F., Proux C., Hwang J., Coppée J.Y., Bahn Y.S. & **Janbon G.** (2016) Intron retention-dependent gene regulation in *Cryptococcus neoformans. (submitted*

Scientific or technical background required for work programs

The candidate should be interested in genetics and molecular biology. An interest in bioinformatics would be a plus.

Institut Pasteur Fall 2016

Title of the work program 2:

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 Master 1 project will be to improve TCR lentivectors by inserting an miRNA specific for endogenous TCR chains. This approach should optimize the expression level of the vectorized TCR, by avoiding competition from endogenous TCR chains and limiting the formation of mixed TCRs heterodimers. The lentivector constructs will be tested for their capacity to suppress endogenous TCR expression by flow cytometry with TCRβ-specific antibodies. 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 programs

- 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.

- Chakrabarti L.A., Boucherie C., Bugault F., Cumont M.C., Roussillon C., Breton G., Patey O., Chêne G., Richert A., Lortholary O., and the ANRS 129 BKVIR-CYTOK STUDY GROUP (2014) Biomarkers of CD4+ T cell activation as risk factors for tuberculosis-associated immune reconstitution inflammatory syndrome. **AIDS** 28 (11):1593–1602 .

- 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.

- Chakrabarti L.A. (2014) The Different Modes of Resistance to AIDS: Lessons from HIV/SIV Controllers and SIV Natural Hosts. In: "**Natural Hosts of SIV: Implications in AIDS**", Edited by Ansari A. A. and Silvestri G., Academic Press, Elsevier, pp. 287-352. ISBN: 978-0-12-404734-1

- 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

Scientific or technical background required for work programs

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 3:

Regulation of the RORγt transcription factor during human T helper cell differentiation

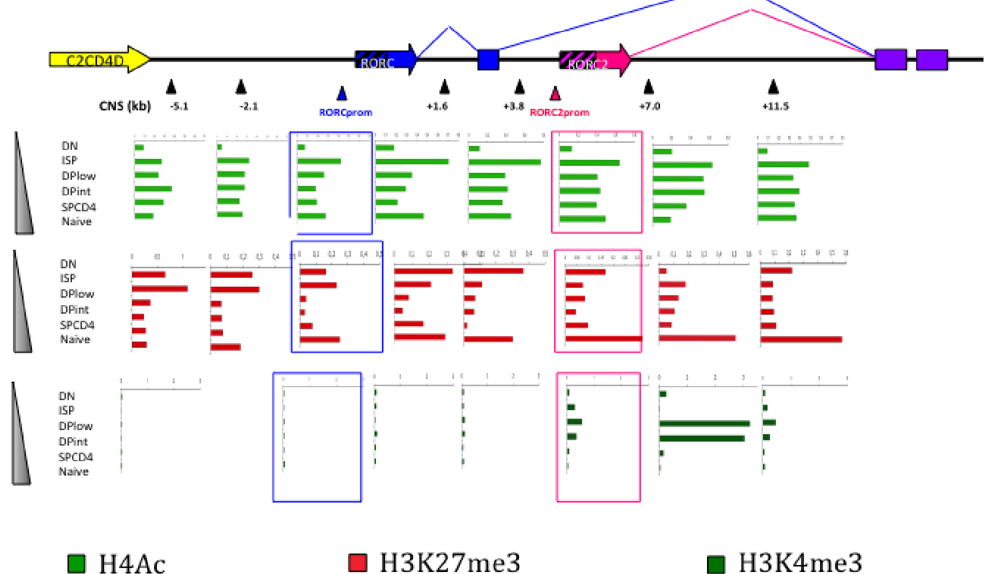
Description of the work program

Generation of inflammatory CD4+ T helper 17 (Th17) cells is highly dependent on the transcription factor retinoid-related orphan receptor, RORγt**.** RORγt was first identified in the mouse as a thymocyte-specific factor and was shown to play a critical role in the regulation of thymopoiesis. In the thymus RORγt is selectively expressed at the double positive stage, and is down-regulated in later stages of thymocyte development, as well as in naïve peripheral CD4+ T cells. RORγt is re-expressed in the periphery during Th17 differentiation. In addition, rare populations of IL-17-producing γδ T cells or innate immune cells can express RORγt.
The molecular mechanisms by which RORγt is transiently expressed during thymopoiesis and re-expressed in selected peripheral lymphocytes are poorly understood. The goal of this project is to define the transcriptional and epigenetic settings that correlate with RORγt expression.

Aim:
In this study we will ask how RORγt expression is regulated at the transcriptional and chromatin level. In particular, we would like to address the following questions:

- Are there specific gene regulatory regions that guide RORγt expression in the thymus and in the periphery?
- What are the transcription factors that guide RORγt expression in these cells?

Preliminary results: epigenetic analysis of the RORC locus during human thymic development
RORγt is expressed from the RORC2 promoter at the RORC locus. We analyzed epigenetic modifications at the RORC locus at different stages of thymocyte development. Our ChIP assays show that H3K4me3, a mark of active promoters, is present specifically at the RORC2 promoter only at stages where RORC2 is expressed, however, the whole RORC locus undergoes extensive remodelling at the transition between the Double Negative (DN) and the Double Positive (DP) stage, assuming a more "permissive" conformation (increased acetylation and decreased H3K27me3). Acetylation of the locus persists in naive cells, possibly allowing more rapid reactivation of RORC2 expression in T cells in the periphery (see Figure).



A similar analysis of epigenetic marks for enhancers (H3K4me1, H3K27ac) has allowed us to identify several regions along the RORC2 locus with potential regulatory function on RORC2 expression. A number of these regions have demonstrated the ability to increase transcription from the RORC2 promoter in reporter gene assays, confirming their role as enhancers.

Work in progress and future plans:

- Epigenetic characterization of the RORC locus in cord blood-derived $CD4^+$ T cells during *in vitro* differentiation to Th17 cells. The *in vitro* differentiation system allows us to analyze the role of selected signalling pathways initiated by TCR or cytokine stimulation on the chromatin conformation at the RORC locus, by testing the impact of addition of selected cytokines, and of specific signalling inhibitors on RORC2 expression and locus remodelling. This approach has lead to the identification of the NFAT pathway as an important factor in RORC2 induction.
- Identification of transcription factors (TF) important for RORC2 induction. The experiments described above will also provide indications about the TF possibly involved in RORC2 induction. We will perform ChIP experiments to test binding of these factors to the RORC locus, and knockdown experiments (siRNA) to test their role in RORC2 induction and chromatin remodelling of the RORC locus. To identify additional factors that may be involved in RORC2 regulation, we have searched for TF and chromatin regulators that are differentially expressed in cells that transcribe versus cells that do not transcribe RORγt. To this end we have compared the transcriptomes of various thymocyte populations, naïve, and IL-17- or IFNγ-producing $CD4^+$ T cells.
- Functional analysis of putative RORC2 enhancers. The activity of genomic regions with putative regulatory function identified with the analysis of epigenetic marks are being tested by gain of function studies, using an in vitro luciferase reporter gene assay to test the ability of these regions and of selected TF to activate transcription.

Tutor/supervisor

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

1. Füzesi-Levi M.G., Ben-Nissan G., Bianchi E., Zhou H., Deery M., Lilley K., Levin Y., Sharon M.. Dynamic regulation of the COP9 signalosome: Insights into the spatial localization and subunit versatility of the complex. *Molecular Cellular Biology,* 34(6):1066-76, 2014

2. M. Hasan, B. Beitz, V. Rouilly, V. Libri, A. Urrutia, D. Duffy, L. Cassard, J. P. Di Santo, E. Mottez, L. Quintana-Murci, M. L. Albert, L. Rogge, Milieu Interieur Consortium, Semi-automated and standardized cytometric procedures for multi-panel and multi-parametric whole blood immunophenotyping. *Clinical Immunology* 157, 261-276 (2015).

3. D. Duffy, V. Rouilly, V. Libri, M. Hasan, B. Beitz, M. David, A. Urrutia, A. Bisiaux, S. T. Labrie, A. Dubois, I. G. Boneca, C. Delval, S. Thomas, L. Rogge, M. Schmolz, L. Quintana-Murci, M. L. Albert, C. Milieu Interieur, Functional Analysis via Standardized Whole-Blood Stimulation Systems Defines the Boundaries of a Healthy Immune Response to Complex Stimuli. *Immunity* 40, 436-450 (2014).

4. S. Dong, S. Maiella, A. Xhaard, Y. Pang, L. Wenandy, J. Larghero, C. Becavin, A. Benecke, E. Bianchi, G. Socie, L. Rogge, Multiparameter single-cell profiling of human CD4+FOXP3+ regulatory T-cell populations in homeostatic conditions and during graft-versus-host disease. *Blood* 122, 1802-1812 (2013).

5. M. Coffre, M. Roumier, M. Rybczynska, E. Sechet, H. K. Law, L. Gossec, M. Dougados, E. Bianchi, L. Rogge, Combinatorial control of Th17 and Th1 cell functions by genetic variations in genes associated with the interleukin-23 signaling pathway in spondyloarthritis. *Arthritis and Rheumatology* 65, 1510-1521 (2013).

Scientific or technical background required for work programs

A good understanding of basic immunology and molecular biology (gene transcription) preferred.

Title of the work program 4:

Nuclear targets of an intracellular bacterium: identification using proximity assays

Description of the work program

Our laboratory studies the interactions between intracellular bacteria and their host cells, with the aim of finding novel targets to fight infection, as well as to gain knowledge on basic cell biology processes. We focus on one intracellular bacterium called *Chlamydia*. Species pathogenic to humans, mainly *Chlamydia trachomatis* and *Chlamydia pneumoniae*, cause a number of diseases, including trachoma, pelvic inflammatory disease and pneumonia.

To survive and multiply in the host, *Chlamydia* interfere with many cellular functions, mainly through the action of proteins secreted into the host cytoplasm, called effector proteins. Once in the cytoplasm, bacterial effectors target a variety of host processes. We have recently performed a screen to uncover chlamydial proteins with nuclear tropism, which raised several candidates.

The internship will aim at the identification of the targets of these bacterial proteins in the nucleus by adapting two proximity assays to *C. trachomatis* infection. First, we will use the DamID technology, in which genomic regions that are in molecular contact with a nuclear protein of interest are tagged *in vivo* with adenine-6-methylation [1]. Using this assay we will discover if the effector localizes to specific regions of the chromatin, and identify those. Complementary to this approach, we will use BioID to identify proteins that are in close proximity to the nuclear effector. This technique uses a ligase activity to covalently tag proteins in proximity of given bait with biotin [2]. Coupled to proteomics, this strategy will identify proteins that are in proximity with the nuclear effector. Ideally, both assays will be applied in infected cells, using *C. trachomatis* transformed with a nuclear effector in fusion with the methylase or biotin ligase activity. Expression of these fusion proteins directly in mammalian cells by transfection will be used as an alternative approach, in case their expression in bacteria fails.

The molecular tools developed during the internship will be tested on the chlamydial nuclear effector NUE [3], as a proof of principle, as our lab has identified some of its targets using an alternative strategy. They will then be applied to the functional study of other nuclear effectors of *C. trachomatis*, possibly within the frame of a PhD thesis.

1. van Steensel B, Henikoff S (2000) Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. Nat Biotechnol 18: 424-428.
2. Roux KJ, Kim DI, Raida M, Burke B (2012) A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J Cell Biol 196: 801-810.
3. Pennini ME, Perrinet Sp, Dautry-Varsat A, Subtil A (2010) Histone Methylation by NUE, a Novel Nuclear Effector of the Intracellular Pathogen *Chlamydia trachomatis*. PLoS Pathog 6: e1000995.

Tutor/supervisor

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

Pennini, M.E., Perrinet S., Dautry-Varsat A., and Subtil A. (2010) Histone methylation by NUE, a novel nuclear effector of the intracellular pathogen *Chlamydia trachomatis PLoS Pathog*: 6, e1000995

Furtado, AR, Essid M, Perrinet S, Balañá ME, Yoder N, Dehoux P and Subtil A (2013) The chlamydial OTU-domain like protein ChlaOTU is an early type III secretion effector targeting ubiquitin and NDP52 *Cellular Microbiol.* 15 2064

Boncompain G., Müller C., Meas-Yedid V., Schmitt-Kopplin P., Lazarow P.B. and Subtil A. (2014) The intracellular bacteria Chlamydia hijack peroxisomes and utilize their enzymatic capacity to produce bacteria-specific phospholipids PLoS One 2014;9(1): e86196

Gehre L., Gorgette O., Prévost M-C., Ducatez M., Ball S.G. and Subtil A. (2016) Sequestration of host metabolism by an intracellular pathogen *eLife* 10.7554/eLife.12552

Vromman F., Perrinet P, Gehre L, and Subtil A. The ESCRT machinery is targeted by a domain conserved in several effector proteins of *Chlamydiaceae*, and is dispensable for bacterial growth *in vitro. In revision in Frontiers in Cellul. Inf. Microbiol.*

Scientific or technical background required for work programs

The student will be highly motivated, hard working, and with a good background in cell biology. Not particular technical background is required.

Title of the work program 5:

An archaeon at the origin of eukaryotes? Phylogenomics approaches to investigate one of the major transitions in the history of life.

Description of the work program

The origin of eukaryotes is one of the most important and yet open issues of evolutionary biology (Poole and Gribaldo 2014 CSHP Biol). Recently, phylogenomic analyses have suggested that the lineage that led to the first eukaryotic cell might have arisen from within the Archaea (Williams et al. 2013 Nature; Spang et al. 2015 Nature; Raymann et al. 2015 PNAS). This is consistent with the stunning number of novel eukaryotic-like characters that have been discovered in various archaeal clades following the availability of genomic sequences from a growing sampling of their diversity. The possibility of obtaining genomic data from uncultured microorganisms (Rinke et al. 2013 Nature) is in fact producing a real momentum in the field of the Archaea, whose tree is rapidly filling up with many new branches providing unprecedented opportunities for evolutionary studies (Brochier-Armanet et al. 2011 Curr Op Microbiol; Castelle et al. 2015 Curr Biol).

For this stage, the student will carry out a thorough phylogenomic analysis of archaea/eukaryotes relationships. This will involve mining novel archaeal genomes to investigate the distribution of specific eukaryotic characters of interest, such as for example those involved in potential cytoskeleton functions and cell division. Accurate phylogenetic reconstruction will allow understanding their origin and evolutionary history in both archaea and eukaryotes. These analyses will also provide key information on the potential function of eukaryotic-like cellular systems in the archaeal domain, which may open the way for further experimental testing.

During her/his stage, the candidate will acquire the theoretical and methodological bases of phylogenomics, and increase her/his general culture on the diversity and evolution of the Archaea and their relationships with eukaryotes.

References:

-Brochier-Armanet C, Forterre P, Gribaldo S (2011) Phylogeny and evolution of the Archaea : one hundred genomes later. Curr Opin Microbiol. 14(3):274-81.
-Castelle CJ et al. (2015) Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling. Curr Biol.16;25(6):690-701.
-Raymann K, Brochier-Armanet C, Gribaldo S (2015) The two-domain tree of life is linked to a new root for the Archaea. *Proc Natl Acad Sci* U S A. 112(21):6670-5.
-Poole AM and Gribaldo S (2014) Eukaryotic origins: when and how was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology* 6(12):a015990.
-Rinke C et al. (2013) Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499(7459):431-7.
-Spang et al. (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 14;521(7551):173-9.
-Williams TA, Foster PG, Cox CJ, Embley TM (2013) An archaeal origin of eukaryotes supports only two primary domains of life. Nature 504(7479):231-6.

Tutor/supervisor

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

-Raymann K, Brochier-Armanet C, Gribaldo S (2015) The two-domain tree of life is linked to a new root for the Archaea. *Proc Natl Acad Sci* U S A. 112(21):6670-5.

-Raymann K, Forterre P, Brochier-Armanet C, Gribaldo S (2014) Global phylogenomic analysis disentangles the complex evolutionary history of DNA replication in archaea. *Genome Biol Evol.* 6(1):192-212.

-Poole AM and Gribaldo S (2014) Eukaryotic origins: when and how was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology* 6(12):a015990.

-Borrel G, O'Toole P, Peyret P, Brugère JF, and Gribaldo S (2013) Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. *Genome Biol Evol.* 5(10):1769-80.

-Brochier-Armanet C, Forterre P, Gribaldo S (2011) Phylogeny and evolution of the Archaea: one hundred genomes later. *Curr Opin Microbiol.* 14(3):274-81.

-Criscuolo A and Gribaldo S (2011) Large-scale phylogenomic analyses indicate a deep origin of primary plastids within Cyanobacteria. *Mol Biol Evol.* 28(11):3019-32.

-Gribaldo S, Poole AM, Daubin V, Forterre P, Brochier-Armanet C (2010) The origin of eukaryotes and their relationship with the Archaea: are we at a phylogenomic impasse? *Nat Rev Microbiol* 8(10):743-52.

Scientific or technical background required for work programs

The candidate should have a strong interest in evolution and genomics. Previous experience with sequence analysis is preferred.

Title of the work program 6:

Cell Biology of Notch in *Drosophila*

Description of the work program

During development, cell-cell communication regulates cell fate. Notch is a receptor that mediates cell-cell communication. This receptor is conserved in all Metazoan species. In humans, perturbations of Notch signaling underlie various adult-onset diseases (notably cancer but laos hypertension, multiple sclerosis etc...). This receptor was first identified in *Drosophila* and is still best studied in this model organism.

Notch receptors can be described as membrane-tethered transcriptional activators that are are released (activated) in response to mechanical pulling of its extracellular ligand-binding domain (1). In the absence of ligands, Notch receptors are in an auto-inhibited state. Ligand binding combined with mechanical puling triggers a conformational change rendering accessible an extracellular cleavage site. Ligand- and force-dependent cleavage of Notch generates a proteolytic fragment that is further processed by ©-secretase to release an activated intracellular form of Notch that localizes to the nucleus and regulates gene expression.

Extensive studies in model organisms, notably *Drosophila*, have identified all core components and many regulators of Notch. Yet, a simple basic question remains: where is Notch activated at the subcellular level? Obviously, Notch should be activated at membrane sites where both receptors and ligands accumulate and interact. However, activation may not necessarily occur whenever and wherever Notch and ligands localize and interact. Indeed, a ligand-dependent mechanical stimulus can only be exerted when ligands at the surface of a given cell interact in *trans* (across the extracellular space) with receptors at the surface of another cell. So, when receptors interact with their ligands in *cis* (within the same cell), no force can be transmitted. Thus, receptor-ligand *cis*-interactions compete with and inhibit *trans*-interaction. This regulatory process is known as 'cis-inhibition' (2). Therefore, the relative levels of the receptor and of its ligands at a given membrane domain likely contribute to the activation/inhibition of Notch. Since the presence of both ligands and receptors cannot reliably predict activation, a major objective (and challenge) is to develop strategies to determine when, where and how Notch is activated *vs* inhibited.

To study the dynamics of Notch activity in *Drosophila*, our laboratory has developed fluorescent Notch receptors (3). Using these innovative tools, we can measure the activity and trafficking of Notch in real time with single cell resolution in the living flies. In the context of the asymmetric cell division of a neural precursor cell, we have shown that Notch is activated in one only of the two daughter cells soon after division. We have also shown that Notch is inhibited and trafficked towards late endosomes in the other daughter cell (3,4).

The proposed project has one big aim, i.e. determining where at the subcellular level Notch is activated in the context of the living organism. Cutting-edge microscopy-based approaches,

1(placements 2016/2017)

including optogenetics (5) will be used to precisely map the subcellular origin (membrane domain and/or endocytic compartment) of the pool of activated Notch that can be found in the nucleus.

Having established when and where Notch is activated, we propose to investigate whether trans-activation and cis-inhibition correlates with the subcellular distribution of the receptor and its ligands and explore the role of intracellular trafficking of both receptors and ligands in this regulation.

This cell and developmental biology project will involve cutting-edge approaches in fly genetics, genome engineering and live imaging by confocal microscopy. The project will be supervised by F. Schweisguth, head of the lab, and M. Trylinski, PhD student working on the Cell Biology of Notch.

1. Kopan, R., & Ilagan, M. X. G. (2009).
The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism. Cell, 137(2), 216–233.
2. del Álamo, D., Rouault, H., & schweisguth, F. (2011).
Mechanism and Significance of cis-Inhibition Review in Notch Signalling. Current Biology 21(1), R40–R47.
3. Couturier, L., Vodovar, N., & schweisguth, F. (2012).
Endocytosis by Numb breaks Notch symmetry at cytokinesis. Nature Cell Biology, 14(2), 131–139.
4. Couturier, L., Trylinski, M., Mazouni, K., Darnet, L., & Schweisguth, F. (2014).
A fluorescent tagging approach in Drosophila reveals late endosomal trafficking of Notch and Sanpodo. The Journal of Cell Biology, 207(3), 351–363.
5. Zhang, K., & Cui, B. (2015).
Optogenetic control of intracellular signaling pathways. Trends in Biotechnology, 33(2), 92–100.

Tutor/supervisor

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

• L. Couturier et al. (2014) A fluorescent tagging approach in Drosophila reveals late endosomal trafficking of Notch and Sanpodo. The Journal of Cell Biology, 3, 351-63

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

• L. Couturier et al. (2012) Endoyctosis by Numb breaks Notch symmetry at cytokinesis. Nature Cell Biology, 14, 131-9

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, Microscopy (live imaging and image analysis) and Genetics/Development is needed.

Title of the work program 7:

Project 1. The tumor suppressor *Adenomatous polyposis coli* as a regulator of anti-tumor immunity

Project 2. Role of inositol 5’ phosphatases in immune synapse organization and T cell activation

Description of the work program

T cells are key effectors of the immune system in higher organisms. In order to perform their functions, they need to be activated by recognizing molecular fragments derived from pathogens (antigens) displayed by specialized antigen-presenting cells. This event requires the generation of organized cell-cell contacts between T cells and antigen-presenting cells, named immunological synapses. These cellular contacts control initial T cell activation, leading to their proliferation and differentiation, but also enable triggering of T cell effector functions, like polarized secretion of cytokines or cytotoxic granules. Our laboratory is interested in understanding how immunological synapses are organized at the molecular level and how they control T cell functions. In particular, we investigate the role of membrane receptors, intracellular signaling molecules, the actin and microtubule cytoskeleton and intracellular vesicle traffic in the formation of immunological synapses and in T cell activation. We are also interested in understanding how alterations of these cellular mechanisms impact on immune responses and contribute to specific pathologies such as cancer or viral infections.
We propose a 6-month training opportunity in the framework of either of the research projects outlined below (the candidate can choose the project).
Project 1.
Adenomatous polyposis coli (APC) is a multifunctional protein regulating cell polarity, cell growth and differentiation in multiple cell types. APC acts as a tumor suppressor and its mutations are involved in a familial form of intestinal polyposis. These mutations alter intestinal epithelial cells, leading to the development of numerous polyps that later evolve into colorectal cancer. APC mutations are also found in the majority of sporadic colorectal cancers. APC also regulates cytoskeleton organization and cell migration, which are altered in metastatic cells, working in concert with other polarity regulators such as Dlg1 and cytoskeleton proteins as ezrin.
Although the role of APC in immune cells is unclear, it has been reported that regulatory T cell (Treg) function is altered in mice bearing mutation of APC, thus predisposing to the development of intestinal polyps and cancers. Our recent results suggest that defective Treg function is due, at least in part, to APC-dependent alterations of the microtubule network which, in turn, impair nuclear translocation and activity of the key transcription factor NFAT.

These results and other available data suggest that APC mutations may also affect other T cell subsets, in particular cytotoxic T cells (CTLs), which are essential for killing tumor cells. For instance, defective NFAT-dependent transcription may alter differentiation of CTLs, e.g. reducing their number and/or altering their phenotype. Moreover, APC-dependent defects of the microtubule network may affect cell polarity, thus impairing the ability of CTLs to interact with cancer cells, to secrete cytotoxic granules and kill the tumor cells. Finally, since APC control migration of multiple cell types, its mutations may alter CTL migration into tumors. These defects would hamper anti-tumor activity of CTLs, hence favoring tumor development.
We propose a training period within a project aimed at studying the role of APC in normal T cell function and in anti-tumor immune responses. Specific points to be addressed by the trainee include the following: 1. Investigate the role of APC in the differentiation of CTLs; 2. Analyze the involvement of APC in CTLs effector functions; 3. Address the role of APC in CTL motility *in vitro*. Human CTLs will be generated by *in vitro* stimulation of blood leukocytes from healthy donors and APC expression will be inhibited by RNA interference. Phenotypic studies will be performed by flow cytometry analysis of CTL receptors and differentiation markers. Confocal, TIRF or high-resolution microscopy will be used to study the interactions of CTLs with target cells and the release of cytotoxic granules. Functional assays will include *in vitro* measurement of cellular cytotoxicity, transmigration and motility of CTLs.

Project 2.

Membrane phosphoinositides play a key role in T cell activation since they regulate cytoskeleton rearrangements, polarized vesicular traffic and clustering of receptors and signaling proteins. The local concentration of these lipids is controlled by the opposing action of phosphoinositide kinases and phosphatases, which are part of the T cell antigen receptor signal transduction cascades. We recently initiated a project to study the potential role of two phosphoinositide phosphatases, OCRL and INPP5B, in immunological synapse formation and T cell activation. We obtained evidence indicating that these enzymes link TCR signaling to actin cytoskeleton and cell membrane reorganization, thus suggesting their implication in immunological synapse formation.

In order to confirm and extend these results, we plan to produce and characterize T cell lines deficient for INPP5B. The main objective of this work program will be to generate INPP5B knockout cells using the CRISPR/Cas9 technology. The trainee will be in charge of the transfection of cell lines with Cas9 nuclease and suitable targeting RNA sequences ("guide RNAs"), selection and validation of mutated cell lines and initial phenotypic and functional analyses of selected clones. These analyses will be performed using different approaches, including flow cytometry, confocal microscopy and T cell activation assays *in vitro* (e.g. assessing the production of specific cytokines or analyzing the activation of specific T cell signaling pathway).

Tutor/supervisor

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

Niedergang, F., V. Di Bartolo, and A. Alcover, *Comparative Anatomy of Phagocytic and Immunological Synapses.* Front Immunol, 2016. **7**:18.

Soares, H., R. Henriques, M. Sachse, L. Ventimiglia, M.A. Alonso, C. Zimmer, M.I. Thoulouze, and A. Alcover, *Regulated vesicle fusion generates signaling nanoterritories that control T cell activation at the immunological synapse.* Journal of Experimental Medicine, 2013. **210**:2415-2433.

Lasserre, R., C. Cuche, R. Blecher-Gonen, E. Libman, E. Biquand, A. Danckaert, D. Yablonski, A. Alcover, and V. Di Bartolo, *Release of serine/threonine-phosphorylated adaptors from signaling microclusters down-regulates T cell activation.* Journal of Cellular Biology, 2011. **195**:839-853.

Lasserre, R., S. Charrin, C. Cuche, A. Danckaert, M.I. Thoulouze, F. de Chaumont, T. Duong, N. Perrault, N. Varin-Blank, J.C. Olivo-Marin, S. Etienne-Manneville, M. Arpin, V. Di Bartolo, and A. Alcover, *Ezrin tunes T-cell activation by controlling Dlg1 and microtubule positioning at the immunological synapse.* EMBO Journal, 2010. **29**:2301-2314.

Roumier, A., J.C. Olivo-Marin, M. Arpin, F. Michel, M. Martin, P. Mangeat, O. Acuto, A. Dautry-Varsat, and A. Alcover. The membrane-microfilament linker ezrin is involved in the formation of the immunological synapse and in T cell activation. Immunity 2001. **15**:715-728.

(see also https://research.pasteur.fr/en/team/lymphocyte-cell-biology/)

Scientific or technical background required for work programs

The ideal candidate should have some background in immunology and/or cell biology. Practical experience in cell culture, flow cytometry, western blotting and confocal microscopy or other imaging techniques would be very useful.

Title of the work program 8:

INTERACTANTS OF PBP1 IN PEPTIDOGLYCAN REMODELLING COMPLEXES OF *H. pylori*

Description of the work program

Project

Peptidoglycan is a meshwork of glycan strands cross-linked by short peptides which surrounds the cytoplasmic membrane of bacteria. It makes them resistant to turgor pressure and gives them their shapes. To accomodate bacterial growth and division, it is constantly hydrolyzed and repolymerized by a large number of enzymes, among which penicillin-binding proteins (PBP) play a major role. As a specific character of bacteria, peptidoglycan is a major antibiotic target. We are using *Helicobacter pylori* as a model to study peptidoglycan remodeling for several reasons. *H. pylori* is an important human pathogen colonizing nearly half of the human population and it is involved in ulcer and gastric cancer. It has a minimal repertoire of peptidoglycan remodeling enzymes (in particular it has only 3 PBPs). Finally, its shape has been proposed as an important determinant of its pathogenesis, and under laboratory conditions it undergoes a transition from spiral to coccoid form in stationary phase. This project aims at finding partners of the central peptidoglycan synthesizing enzyme, PBP1, in order to better understand these changes and find compounds able to disrupt peptidoglycan remodelling complexes.

Peptidoglycan remodelling appears to be highly dynamic and to involve different complexes according to the stage of the cell-cycle (1). The centerpiece of these complexes is composed of 2 PBPs: one (PBP1 in *H. pylori*) has two activities: glycosyltransferase (GT, elongating the glycan strand) and transpeptidase (TP catalysing the formation of peptide cross-links); the other (either PBP2 or PBP3 in *H. pylori*) has a transpeptidase activity only. Other proteins which can be present in these complexes are cell morphogenesis proteins like Mre or Fts proteins, or enzymes that hydrolyze either the glycan strands or the cross-linking peptides, which are probably cutting the old peptidoglycan to insert the newly synthesized one. In *H. pylori*, we have already identified several peptidoglycan hydrolases and observed a physiological complex between PBP2 and the cell-shape protein MreC, but we still lack a systematical analysis of the interactants of PBP1. Note that this protein is the only one expected to participate to all types of peptidoglycan synthesizing complexes (those with PBP2, probably mediating cell elongation, as well as those with PBP3, involved in cell division).

We intend to use tandem affinity purification to fish out the interactants of PBP1 in *H. pylori* at different stages of its growth. Since the targeted protein assemblies are dynamic, there is a risk that they fall apart during purification, therefore we will use chemical cross-linking agents to freeze the complexes and keep the interacting proteins together. To achieve high specificity, we will purify the cross-linked complexes in denaturing conditions by using affinity tags that withstand these conditions (like the His-tag and the biotinylation tag (2)). Proteins that will copurify with the tagged PBP1 will be identified by mass spectrometry. We will validate potential interactants by doing the reverse tandem affinity purification (i.e. tagging the interactant and checking whether PBP1 copurifies with it). We will determine whether the genes encoding the interactant proteins are essential or not by creating deletion mutants or conditional mutants. We will study the mutants in terms of bacterial growth and cell shape by microscopy to determine the physiological role of these proteins. To gain a more precise insight into their activity, we will analyze the architecture of the peptidoglycan of the mutants by mutanolysin digestion and HPLC separation of the muropeptides.

We expect to identify potential determinants of cell shape, and PBP1-interactant complexes with a dual interest: be a subject for further biochemical and structural studies leading to a better understanding of peptidoglycan synthesis complexes in *H. pylori*, and provide testable targets for alternative antibacterial compounds.

1. Typas A, Banzhaf M, Gross CA, & Vollmer W (2012) From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nature reviews. Microbiology* 10(2):123-136.

2. Xu X, *et al.* (2010) The tandem affinity purification method: an efficient system for protein complex purification and protein interaction identification. *Protein Expr Purif* 72(2):149-156.

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

El Ghachi, M. P.-J. Matteï, **C. Ecobichon**, A. Martins, S. Hoos, C. Schmitt, F. Colland, C. Ebel, M.-C. Prévost, F. Gabel, P. England, A. Dessen & **I.G. Boneca**. 2011. Characterization of the elongasome core PBP2:MreC complex of *Helicobacter pylori. **Mol Microbiol**.* 82. (1):68-86.
Roure, R., M. Bonis, C. Chaput, **C. Ecobichon, A. Mattox**, C. Barrière, N. Geldmacher, S. Guadagnini, C. Schmitt, M.-C. Prévost, A. Labigne, S. Backert, R.L. Ferrero & **I.G. Boneca**. 2012. Peptidoglycan maturation enzymes affect flagellar functionality in bacteria. ***Mol Microbiol***. 2012 Sep 21. Doi: 10.111/mmi.12019.
Bonis, M., C. Ecobichon, S. Guadagnini, M.-C. Prévost & **I.G. Boneca**. 2010. A M23B- family metallopeptidase of *Helicobacter pylori* required for cell shape, pole formation and virulence. ***Mol. Microbiol***. 78. (4):809-819.

Scientific or technical background required for work programs

Basic knowledge in genetics and molecular biology

Title of the work program 9:

CCR5 conformations and HIV-1 entry

Description of the work program

HIV entry into target cells requires the interaction between HIV envelope glycoproteins (gp120/gp41) and two cellular receptors, CD4 and a chemokine receptor CCR5 and/or CXCR4. These receptors belong to the class A of the G-protein coupled receptors (GPCR) family. GPCRs are able to form dimers or larger oligomers, a process involved in ligand binding, cell signaling, and cell surface expression. However, the role played by CCR5 dimerization in receptor physiology and HIV pathogenesis is still unknown. We recently identified several CCR5 dimerization interfaces, suggesting the existence of multiple CCR5 conformations. To characterize these conformations and their function, we will study how ligands and/or partners will influence the dimeric organization of CCR5. The work program will include molecular biology to perform mutagenesis of the receptor, biochemistry to validate the dimerization interfaces (using covalent intermolecular cross-linking experiments), biophysical approaches to assess receptor dimerization (FRET), and virological approches to quantify HIV-1 infection.

Tutor/supervisor

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

HIV-1 exploits CCR5 conformational heterogeneity to escape inhibition by chemokines. Colin P, Bénureau Y, Staropoli I, Wang Y, Gonzalez N, Alcami J, Hartley O, **Brelot A**, Arenzana-Seisdedos F, Lagane B. Proc Natl Acad Sci U S A. 2013 May 21

Targeting spare CC chemokine receptor 5 (CCR5) as a principle to inhibit HIV-1 entry. Jin J, Colin P, Staropoli I, Lima-Fernandes E, Ferret C, Demir A, Rogée S, Hartley O, Randriamampita C, Scott MG, Marullo S, Sauvonnet N, Arenzana-Seisdedos F, Lagane B, **Brelot A**. J Biol Chem. 2014 Jul 4;289(27):19042-52

Scientific or technical background required for work programs

- Scientific knowledge in biochemistry, cell biology, and physiology of GPCRs.
- Technical background in biochemistry, molecular biology, and cell biology.

Title of the work program 10:

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

Title of the work program 11:

Characterization of *Streptococcus gallolyticus gallo2021-2018* locus encoding bacteriocins, targeting *Enterococcus faecalis*, a prominent member of gut microbiota.

Description of the work program

Streptococcus gallolyticus (also known as *S. bovis* type I) is one of the few opportunistic pathogens, which has been unambiguously linked to colonic malignant diseases. A recent meta review of the epidemiological data showed that 65% of the patients infected with *S. bovis*, who mostly had endocarditis, had concomitant colorectal neoplasia {Boleij, 2011}. Colorectal cancer (CRC) is the third most common cause of cancer mortality in the world, with more than 600 000 deaths every year. CRC is typically a genetic disease that develops over many years via a sequence of genetic changes known as the adenoma-carcinoma sequence. Tumors are more frequent in the distal large intestine, the habitat of a vast and complex community of microorganisms referred as “gut microbiota” whose contribution in the development of CRC is increasing. We aim at answering the following questions: Why a high prevalence of *S. gallolyticus* in the gut of patients with CRC? Is this bacterium a natural predator, or is it a commensal whose colonizing properties are influenced by particular environmental conditions? What are the colon tumor characteristics that can affect colonization by *S. gallolyticus*?

Recent data obtained in the murine model indicate that in the presence of a tumor, *S. gallolyticus* is able to outcompete *Enterococcus faecalis*, a major member of the endogenous microbiota (L. Aymeric, unpublished data). To do so, *S. gallolyticus* secretes small class IIC bacteriocins which are encoded in the locus *gallo_2021-2018*. Blast analyses indicate highest similarity with the highly variable *blp* (bacteriocin-like peptide) locus that has been found in all sequenced strains of *Streptococcus pneumoniae*. This locus controls the regulation and secretion of bacteriocins, small peptides that target other bacteria, which mediate both intra- and inter-specific competition in the human nasopharynx.

The project consists in the detailed characterization of the genetic locus encoding bacteriocins in *S. gallolyticus* UCN34. We will start with the role of *gallo_2021*, *gallo_2020*, and *gallo_2019*, through the construction of in-frame deletion mutants in *S. gallolyticus* strain UCN34 and testing the role of individual mutants in the killing of *E. faecalis in vitro*. We will also seek to determine the mode of action of these bacteriocins, their regulation, their secretion and how the producing strains are immune to their action. To identify the environmental signal(s) turning ON the bacteriocins production, the promoter of this locus has been placed in front of the gene encoding the green fluorescent protein. Preliminary results showed that Tween 20, a detergent like molecule is able to trigger this promoter ON during late-exponential phase.

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

1. Martins M, Aymeric L, du Merle L, Danne C, Robbe-Masselot C, Trieu-Cuot P, Sansonetti P, Dramsi S*. *Streptococcus gallolyticus* Pil3 pilus is required for adhesion to colonic mucus and for colonization of mouse distal colon. J. Infect. Dis. 2015 Nov 15;212(10):1646-55.

2. Danne C, Dubrac S, Trieu-Cuot P, Dramsi S*. Single cell stochastic regulation of pilus phase variation by an attenuation-like mechanism. PLoS Pathog. 2014 Jan; 10 (1):e1003860.

3. Danne C, Guérillot R, Glaser P, Trieu-Cuot P, Dramsi S*. Construction of isogenic mutants in Streptococcus gallolyticus based on the development of mobilizable vectors. Res. Microbiol. 2013 164(10):973-8.

4. Danne C, Dramsi S*. Pili of gram-positive bacteria: roles in host colonization. Res. Microbiol. 2012 163(9-10):645-58. Review

5. Danne C, Entenza JM, Mallet A, Briandet R, Débarbouillé M, Nato F, Glaser P, Jouvion G, Moreillon P, Trieu-Cuot P, Dramsi S*. Molecular characterization of a Streptococcus gallolyticus genomic island encoding a pilus involved in endocarditis. J Infect Dis. 2011 Dec 15; 204(12):1960-70.

Scientific or technical background required for work programs

The applicant should be familiar with basics in molecular cloning techniques and skills in microbiology techniques will be a plus.

Title of the work program 12:

Deciphering the genetic determinants responsible for Φ13 prophage mobility in *S. aureus*

Description of the work program

Staphylococcus aureus, a major human pathogen, causes life threatening hospital- and community-acquired infections. Yet *S. aureus* is also a commensal, asymptomatically colonizing the interior nares of about a third of the population. The switch between commensal and pathogen lifestyles remains poorly understood. Beyond transcriptomic adaptation, reversible genome modification through prophage induction could play an important role in the transition from commensal to pathogen. The *hlb*-converting phage Φ13 carries genes involved in escape from the innate immune response (*sak*, *scn* and *chp*) and is inserted within the coding region of the β-hemolysin, a host-damaging toxin. Our data suggest that prophage mobilization occurs during infection and that it is involved in *S. aureus* virulence.

In order to understand how the bacteria switch from a lysogenic form to a free phage form, we are interested in deciphering the regulatory circuit(s) leading to Φ13 excision.

The proposed project concerns the study of the Φ13 regulatory locus. We have identified at least three potential regulators encoded by the phage genome. We now need to understand the role of each of these regulators. For this purpose, we propose to generate in frame deletions of the coding sequences for each of the regulators and to follow Φ13 excision either by phenotypic characterization (recovery of a β-hemolytic halo on sheep blood agar) or by quantifying prophage excision using quantitative real time PCR (qRT-PCR). Furthermore, within the Φ13 genome, we have identified two genes encoding for the putative Φ13 phage integrase and excisionase. We will test the role of the three putative regulators on the expression of these genes either by fusing their promoter to a reporter gene (GFP) or by qRT-PR. Lastly, we would like to understand how these regulators can regulate prophage excision functions. For this purpose, we plan to measure the expression levels of these regulatory genes and to perform bacterial two-hybrid experiments in order to map physical interactions between these regulators.

Tutor/supervisor

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

1. Delaune A, Poupel O, Mallet A, Coic YM, Msadek T, & Dubrac S (**2011**) Peptidoglycan crosslinking relaxation plays an important role in *Staphylococcus aureus* WalKR-dependent cell viability. *PLoS One* 6(2):e17054.
2. Delaune A, Dubrac S, Blanchet C, Poupel O, Mader U, Hiron A, Leduc A, Fitting C, Nicolas P, Cavaillon JM, Adib-Conquy M, & Msadek T (**2012**) The WalKR System Controls Major Staphylococcal Virulence Genes and Is Involved in Triggering the Host Inflammatory Response. *Infect. Immun.* 80(10):3438-3453.
3. Lioliou E, Fechter P, Caldelari I, Jester BC, Dubrac S, Helfer AC, Boisset S, Vandenesch F, Romby P, & Geissmann T (**2016**) Various checkpoints prevent the synthesis of Staphylococcus aureus peptidoglycan hydrolase LytM in the stationary growth phase. *RNA Biol*:1-14.
4. Poupel O, Moyat M, Groizeleau J, Antunes LC, Gribaldo S, Msadek T, & Dubrac S (**2016**) Transcriptional Analysis and Subcellular Protein Localization Reveal Specific Features of the Essential WalKR System in Staphylococcus aureus. *PLoS One* 11(3):e0151449.

Scientific or technical background required for work programs

Experience in bacterial cultivation, bacterial genetic and basic molecular biology would be appreciated. A theoretical knowledge about qRT-PCR would be a plus.

Title of the work program 13:

Study of the interplay between nucleoporins and chromatin factors to orchestrate HIV-1 replication.

Description of the work program

NPCs are stable structures with specific functions in nuclear transport, genome organization, genome stability and gene expression regulation. Non dividing cells are the major target of HIV-1, thus its passage through the NPC is a key step for viral replication.
Several studies, including ours, investigated the mechanistic requirements of nucleoporins (Nups) in the HIV-1 life cycle. However, the study of the individual role of Nups in HIV-1 infection is complicated, because many Nups act as scaffold for others, thus their structural association is a major difficulty in determining the role of individual Nups in HIV-1 infection.
Our research is currently focused on the interplay between HIV-1 components, NPC and chromatin. We aim to unravel the link between nuclear import, chromatin organization and transcriptional regulation. Interestingly, some nucleoporins interact with actively transcribed chromatin which is also the target of HIV-1 integration.

We aim to exploit Nups, to identify complexes composed by the target Nups, HIV-1 components, chromatin and nuclear cellular factors. These complexes may work in concert with Nups to orchestrate the viral replication underneath the nuclear pore complex.

In particular, Nup153 has a critical role in HIV-1 nuclear import, however it is still unknown how this Nup leads HIV-1 to use the pore. Besides, nuclear basket Nups bind particular chromatin regions and regulate genes activity, thus, our aim is to understand how Nups, chromatin factors and genes are concerted to orchestrate HIV-1 replication. Nuclear basket Nups may be another “cellular code” for specifying HIV-1 fate through their contacts with the underlying chromatin.
.
For this project, the candidate will benefit of the expertise of the lab in early steps of HIV-1 replication cycle and of the development of innovative and trans-disciplinary approaches.

Overall, this study should add an important piece to our understanding of HIV-1 replication mechanisms and could serve in the development of new antiviral strategies.

Tutor/supervisor

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

- J. Valle-Casuso*, F. Di Nunzio*, N. Reszka, M. Lienlaf, N. Arhel, P. Perez, A.L. Brass, F. Diaz-Griffero : TNPO3 Binds the HIV-1 Assembled Capsid and Assists HIV-1 Replication After Nuclear Import but Prior to Integration **J Virol.** 2012.

- M. Lelek*, F. Di Nunzio*, R. Rodriguez, P. Charneau, N. Arhel, and C. Zimmer : Super-resolution imaging of HIV in infected cells with FlAsH-PALM. **PNAS**, 2012

- F. Di Nunzio, A. Danckaert, T. Fricke, P. Perez, J. Fernandez, E. Perret, P. Roux, S. Shorte, P.Charneau, F. Diaz-Griffero, N. J. Arhel: Human Nucleoporins Promote HIV-1 Docking at the Nuclear Pore, Nuclear Import and Integration. **PLOS ONE**, September 25, 2012.

- F. Di Nunzio✉, T. Fricke, A. Miccio, J.C. Valle-Casuso, P. Souque, P. Perez, E. Rizzi, M. Severgnini, F. Mavilio, P. Charneau and F. Diaz-Griffero✉: Nup153 and Nup 98 bind the HIV-1 core and contribute to the early steps of HIV-1 replication in human lymphocytes. **Virology**, May 2013

- F. Di Nunzio✉**:** New insights in the role of nucleoporins: a bridge leading to concerted steps from HIV nuclear entry until integration. Review, **Virus Research**, 2013

- M. Morchikh, M. Naughtin *, F. Di Nunzio*, J. Xavier, P. Charneau, Y. Jacob and M. Lavigne: TOX4 and NOVA1proteins are partners of the LEDGF PWWP domain and affect HIV-1 replication. **PLOS ONE**, Nov 2013

- M. Lelek*✉, F. Di Nunzio*✉, C. Zimmer✉: FlAsH-PALM: Super-resolution localization microscopy with FlAsH-tetracysteine labeling and statistical analysis of subdiffraction viral morphology. Chapter in **Methods in Molecular Biology**, 2014

- Lelek M, Casartelli N, Pellin D, Rizzi E, Souque P, Severgnini M, Di Serio C, Fricke T, Diaz-Griffero F, Zimmer C, Charneau P, Di Nunzio F✉ Chromatin organization at the nuclear pore favours HIV replication. **Nat Commun**. 2015 Mar 6; **Open Access**

(* equal contribution; ✉ corresponding author)

Scientific or technical background required for work programs

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

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

Title of the work program 14:

Characterization of VC1636 protein and its role in induction of SOS response in *Vibrio cholerae*

Description of the work program

The bacterial SOS response is a global stress response involved in DNA-damage repair. SOS induction takes place when single stranded DNA (ssDNA) is detected in the cell and activates recombination and repair pathways. The SOS response also results in increased mutagenesis, genome rearrangements and the acquisition of resistance to antibiotics. In Escherichia coli it is induced after DNA damage, for example, after treatment with antibiotics that target DNA. Our model is *Vibrio cholerae*, a Gram-negative human pathogen that grows planktonically or as a biofilm on crustacean shells and is able to couple genome plasticity and adaptation in different environments through modulation of the SOS response.
In *Escherichia coli* SOS is induced after DNA damage, for example after treatment with antibiotics that target DNA. Unexpectedly, the SOS response is induced in *Vibrio cholerae* by exposure to sub-inhibitory concentrations (sub-MIC) of different families of antibiotics that do not target DNA, such as aminoglycosides, chloramphenicol and tetracycline. The use of a genetic screen led to the isolation of mutants in which induction of the SOS response by sub-MICs of aminoglycosides is lost. One of these mutants is inactivated for the vc1636 gene, which encodes a putative DNA/RNA helicase. The *E. coli* homologue of this gene, called yejH, is also uncharacterized. We demonstrated that VC1636 over-expression can complement the UV sensitivity of an *E. coli* Δmfd strain; the Mfd protein is involved in the displacement of the RNA polymerase (RNAP) blocked on a DNA lesion.
The purpose of this project is to characterize the protein encoded by the gene vc1636 and to understand the induction of SOS response by antibiotics that do not target DNA in *V. cholerae*.

Tutor/supervisor

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

Baharoglu, Z. and Mazel, D. 2014. SOS, the formidable strategy of bacteria against aggressions. FEMS MICROBIOLOGY REVIEWS

Baharoglu, Z., A. Babosan, and D. Mazel. 2013b. Identification of genes involved in low aminoglycoside-induced SOS response in Vibrio cholerae: a role for transcription stalling and Mfd helicase Nucleic Acids Research, 2013, 1–14 doi:10.1093/nar/gkt1259

Baharoglu, Z., E. Krin, and D. Mazel. 2013a. RpoS Plays a Central Role in the SOS Induction by Sub-Lethal Aminoglycoside Concentrations in Vibrio cholerae. PLoS Genetics, 9(4): e1003421.

Baharoglu, Z. and D. Mazel, 2011. Vibrio cholerae triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance. Antimicrob. Agents Chemother. 55, 2438–2441.

Scientific or technical background required for work programs

We are looking for a student in life sciences or medicine to do a six month internship in bacterial genetics.
We will give preference to students with basic knowledge in microbiology, molecular biology and genetics.
An additional laboratory experience would be appreciated.
Good knowledge of English is required.

Title of the work program 15:

Role of nicotinic genetic variants in the development of Chronic Obstructive Pulmonary Disease (COPD) and lung cancer, *independently* of smoking levels of the subjects.

Description of the work program

There is very robust human genetic evidence confirmed by meta-analysis of ***G***enome-***W***ide ***A***ssociation ***S***tudies (GWAS) that human ***s***ingle ***n***ucleotide ***p***olymorphisms (***SNPs***) in the CHRNA5/CHRNA3/CHRNB4 cluster on chromosome 15q are linked to Chronic Obstructive Pulmonary Disease (COPD) and lung cancer, ***independently*** of smoking levels of the subjects.

Lung cancer is the leading cause of cancer-related mortality across the world with over 1.3 million deaths each year. Lung cancers comprise four major types: 32% adenocarcinoma (AC), 29% squamous cell carcinoma (SCC), 18% small cell carcinoma and 9% large cell carcinoma (*1*). Smoking is associated with 90% of lung cancer. Exposure to tobacco smoke leads to progressive pathological premalignant changes, from basal cell hyperplasia, metaplasia, dysplasia, and carcinoma *in situ* to invasive carcinoma for SCC. Lung AC often occurs near sites of atypical alveolar hyperplasia, and bronchiolo-alveolar carcinoma, a progressive non-invasive tumor of alveolar epithelial cells that may evolve into invasive AC. Additionally, we know that a large majority of patients with lung cancer present with COPD, and that COPD is a major independent risk factor for lung cancer (*2*). Therefore, it has been postulated that there are common complex mechanisms governing the individual risk of developing lung cancer, COPD or both (*3*). However, common molecular risk factors are currently unknown.

Recently, several genome wide association studies (GWAS) identified novel susceptibility loci for lung cancer and for major histological types. Remarkably, these studies found single nucleotide polymorphisms (SNPs) significantly associated with lung cancer risk, located in a region of the long arm of chromosome 15 (15q25). It contains a cluster of three genes coding for nAChR subunits CHRNA5, CHRNA3 and CHRNB4, expressed in neuronal and other tissues (*4*). These subunits will be referred to as □5, □3, and □4, respectively. The same variants were also associated with an increased risk of COPD (*5*). This region is hypothesized to account for 14% of lung cancer cases irrespective of histological subtype. It is a matter of contention as to whether the implication of these genes in the risk of lung cancer is independent of any effect they may have on smoking behaviour, leading to increased exposure to tobacco carcinogens. Indeed, studies have also identified these same genetic variants to be associated, in addition, with nicotine dependence risk (*4*). Since the nAChRs formed by these genes are also expressed in the lung and are activated by nicotine and other carcinogens present in tobacco smoke, it is conceivable to propose that these nicotinic polymorphisms might increase addiction to tobacco and, therefore, expose more airway nAChRs to tobacco carcinogens. However, and very importantly, other studies also report an increased risk of lung cancer even in non-smokers, suggesting the possibility of a direct effect on lung cancer susceptibility independent of smoking habits (*6*).

The aim of the present project is to dissect, using mouse models and human cell cultures and tissues, the mechanisms by which the □5N398 genetic variant leads to the emergence of preneoplastic lesions in the bronchi and of the distal airway epithelium. We will also evaluate the role of this variant in BPCO and cancers induction.

Our in vivo studies will be complemented by an extensive use of human cells. To molecularly dissect the role of variants in the behaviour of airway cells, we used the Zinc Finger Nuclease technology to knock out human □5 subunit expression in the human bronchial epithelial cell line 16HBE (SV40T), which expresses the genotype □5D398/□5N398, and then also create □5N398 and □5D398 subunit-expressing homozygous cell lines. We will compare the epithelial phenotype of these cell lines in terms of proliferation and migration. We will characterize the responses to pharmacological agents in terms of intracellular calcium modulation and the electrophysiological properties of these engineered 16HBE cell lines. These cell lines will also be used for a comprehensive analysis of the transcriptome, phosphoproteome and methylation patterns distinguishing the normal allele from the risk allele.

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

1. S. S. Devesa, F. Bray, A. P. Vizcaino, D. M. Parkin, International lung cancer trends by histologic type: male:female differences diminishing and adenocarcinoma rates rising. *International journal of cancer. Journal international du cancer* **117**, 294 (Nov 1, 2005).
2. A. Punturieri, E. Szabo, T. L. Croxton, S. D. Shapiro, S. M. Dubinett, Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. *J Natl Cancer Inst* **101**, 554 (Apr 15, 2009).
3. A. M. Houghton, M. Mouded, S. D. Shapiro, Common origins of lung cancer and COPD. *Nat Med* **14**, 1023 (Oct, 2008).
4. S. J. Chanock, D. J. Hunter, Genomics: when the smoke clears. *Nature* **452**, 537 (Apr 3, 2008).
5. R. P. Young *et al.*, Lung cancer gene associated with COPD: triple whammy or possible confounding effect? *Eur Respir J* **32**, 1158 (Nov, 2008).
6. K. Shiraishi *et al.*, Contribution of nicotine acetylcholine receptor polymorphisms to lung cancer risk in a smoking-independent manner in the Japanese. *Carcinogenesis* **30**, 65 (Jan, 2009).

Scientific or technical background required for work programs

We are looking for a motivated candidate who wants to become part of an enthusiastic team defining a novel mechanism for BPCO and lung cancer. Experience in cell biology, biochemistry or immunology would be an advantage.

Title of the work program 16:

Requirement of primary cilia in the epicardial lineage for the growth of the heart

Description of the work program

Growth of the heart is required to adapt to the increasing hemodynamic load of the developing organism. The myocardium, the cardiac muscle, is the main tissue of the heart and responsible for the contractile function. However, other cell types, from the epicardial lineage, are required for the growth of the muscle, because they secrete paracrine growth factors or provide irrigation of the muscle via the formation of coronary vessels. The project will address a role of the epicardial lineage in the mechanism of heart growth.

The primary cilium is an organelle present in most cells, as a kind of antennae that integrates mechanical and growth signals (Goetz and Anderson, 2010). For example, it has been shown that the secreted signal Sonic Hedgehog (Shh) requires a primary cilium for its transduction (Huangfu et al., 2003). In the heart, cells of the epicardial lineage are ciliated throughout development and Shh was shown to be required for the formation of coronary vessels (Lavine et al., 2008). However, no malformation of coronary vessels has been so far associated with cilium dysfunction and heart growth defects in this condition have remained poorly characterised.

We have generated, in the mouse model, conditional inactivations of the ciliary gene *Ift20* in different cardiac lineages, leading to heart growth defects. The aim of the Erasmus+ project will be to characterise the role of the epicardial lineage in the heart growth defects of ciliary mutants. Mutants deprived of primary cilia specifically in the epicardial lineage will be studied. Deployment of the epicardial lineage will be mapped, as well as the growth of coronary vessels. The project involves production of embryonic samples and immunostaining of cardiac markers and of potential ciliary signals such as Shh. Reconstruction of the 3D heart shape and coronary vascular tree will be obtained from High Resolution Episcopic Microscopy followed by quantitative image analysis. The student will work with a PhD student for the analysis of mutants and with a physicist post-doc for the quantitative image analysis. In collaboration with J. Vermot (IGBMC, Strasbourg), we will also evaluate how the role of cilia is conserved between the fish and the mouse.

The project is expected to provide novel insight into the mechanisms of heart growth and is relevant to rare genetic diseases, the ciliopathies, which result from a dysfunction of primary cilia. The laboratory is jointly affiliated to the Institut Pasteur and Institut *Imagine* within the Hospital Necker-Enfants Malades, which has expertise in ciliopathies and congenital heart defects.

Tutor/supervisor

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

Asymmetric fate of the posterior part of the second heart field results in unexpected left/right contributions to both poles of the heart, Domínguez JN, Meilhac SM, Bland YS, Buckingham ME, Brown NA, Circ Res. 2012, 111(10):1323-35.

Extracting 3D cell parameters from dense tissue environments: Application to the development of the mouse heart, S. Pop, A. Dufour, J-F. Le Garrec, C. Ragni, C. Cimper, S. Meilhac and J-C. Olivo-Marin, Bioinformatics 2013, 29(6):772-9.

Quantitative analysis of polarity in 3D reveals local cell coordination in the embryonic mouse heart, J-F. Le Garrec, C. Ragni, S. Pop, A. Dufour, J-C. Olivo-Marin, M. Buckingham and S. Meilhac, Development 2013, 140(2):395-404.

Cilia and heart morphogenesis. Diguet N, Meilhac SM, Med Sci (Paris) 2014, 30(11):996-1003.

2015 Patent WO/2015/121323 : Treatment of cardiac diseases with modulators of the Hippo pathway

Scientific or technical background required for work programs

Interest in developmental biology is required, as well as a previous lab experience.

Title of the work program 17:

Investigating the role of the gut microbiota in nicotine addiction-related processes

Description of the work program

Tobacco smoking remains the most preventable cause of morbidity and mortality worldwide. Nicotine is the main psychoactive component of tobacco responsible for its addictive properties, and interacts with the nicotinic acetylcholine receptors (nAChRs). Drug addiction is a psychiatric disorder with complex dynamics, from the initiation of drug taking to the development of the pathology in some individuals. Several risk factors are likely to contribute to the vulnerability to develop addiction. A growing body of evidence shows that there is a bidirectional communication between the brain and the gut microbiota, and that the latter exerts a significant influence upon a set of functions involved in psychiatric disorders, such as depression, autism, and addiction. Alterations in the gut microbiota and in the brain-gut axis have notably been reported during tobacco abstinence. Also, gut hormones have been implicated in the action of several drugs of abuse including nicotine, and were shown to influence the activity of the reward system. However, the role of the gut microbiota in the different processes that contribute to nicotine addiction has never been directly investigated. The objective of the project is to determine the possible influence of the gut microbiota on nicotine addiction-related features. Specifically, we will investigate, in rodent models, whether gut microbiota depletion alters the response of specific brain circuits to nicotine and the appetence for nicotine in several behavioral models that model addiction-related processes.

Tutor/supervisor

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

Besson M, Guiducci S, Granon S, et al. Alterations in alpha5* nicotinic acetylcholine receptors result in midbrain- and hippocampus-dependent behavioural and neural impairments. Psychopharmacology, 2016. 233(18):3297-314.

Besson M, Pelloux Y, Dilleen R, et al. Cocaine modulation of fronto-striatal expression of zif268, D2 and 5-HT2c receptors in high and low impulsive rats. Neuropsychopharmacology, 2013. 38: 1963-1973.

Besson M, Belin D, McNamara R, et al. Dissociable control of impulsivity in rats by dopamine D2/3 receptors in the core and shell subregions of the nucleus accumbens. Neuropsychopharmacology, 2010. 35: 560-9.

Besson M*, Granon S*, Mameli-Engvall M, et al. Long-term effects of chronic nicotine exposure on brain nicotinic receptors. Proc Natl Acad Sci U S A, 2007. 104: 8155-60.

Maskos U, Molles BE, Pons S, et al. Nicotine reinforcement and cognition restores by targeted expression of nicotinic receptors, Nature. 2005. 436: 103-107. Featured as Faculty of 1000 paper.

Scientific or technical background required for work programs

We are looking for an applicant with good knowledge in neuroscience, ideally in behavioral neuroscience especially in the field of rodent models of psychiatric diseases such as drug addiction and/or in immunology with a special interest in the gut microbiota. The applicant should be interested in conducting behavioral testing in rodents.

Title of the work program 18:

Investigating the role of a new virulence locus involved in hypervirulence of *L. monocytogenes*.

Description of the work program

Listeria monocytogenes (*Lm*) is a foodborne bacterium, which pathogenicity results from its capacity to disseminate systemically and induce neurolisteriosis as well as maternal-fetal listeriosis. *Lm* is a model microorganism, which pathogenesis has been extensively studied in *in vitro* and *in vivo* models, using fully characterized reference strains. Our laboratory has completed a large epidemiological and comparative genomics study based on the activities of the National Reference Center Laboratory for *Listeria*. This has led to the identification of *Lm* clonal complexes, as assessed by Multi Locus Sequence Typing (MLST), which are highly associated with neurolisteriosis and maternal-fetal listeriosis (Maury et al, Nat Genet 2016). Moreover, experimental infections in a humanized mouse model of listeriosis developed in our laboratory have shown that the clonal complexes (CCs) most associated with neurolisteriosis and maternal-fetal listeriosis are hypervirulent (CC1, CC4 and CC6). We have investigated the molecular bases of the hypervirulence of these clones, by comparative genomics and mutagenesis studies. We have uncovered a cluster of six genes annotated as a cellobiose-family phosphotransferase (PTS) system, which is specific for CC4. We have determined that this PTS gene cluster contributes to central nervous system and maternal-fetal infection *in vivo*. The main aim of this project will be to determine the function of the PTS cluster and uncover the molecular mechanism involved in its role in virulence.
Members of the PTS family are involved in the transport and phosphorylation of the carbohydrates. They play a major role in carbohydrates metabolism but may also be involved in gene regulation, in particular virulence genes such as *prfA* from *L. monocytogenes.* Using a global metabolomics approach in wild-type and isogenic mutant deleted for the CC4 specific PTS cluster, the candidate will first determine in which steps of the metabolic pathways the PTS cluster is involved. It will allow determining links to pathways involved in *Lm* virulence. By generating individual mutants of the six genes from the PTS cluster, she/he will demonstrate their respective roles in the determined pathway.
Once the metabolic pathways regulated by the PTS cluster determined, she/he will investigate how the external nutriment sources have an impact on the metabolism, and how it may modulate the virulence genes of *Lm*. A particular focus will be made on PrfA, the major transcription activator of *Lm* virulence genes. The candidate will notably determine if the PTS cluster is involved in PrfA regulation, hence in hypervirulence.
This project will allow characterizing one of the molecular mechanisms involved in hypervirulence of CC4, which is highly associated with neurolisteriosis and maternal-fetal listeriosis. It will also allow a better understanding how *Lm* interferes with the outer environment, and how this modulate virulence of *Lm*.

Tutor/supervisor

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

Maury MM, Tsai YH, Charlier C, Touchon M, Chenal-Francisque V, Leclercq A, Criscuolo A, Gaultier C, Roussel S, Brisabois A, Disson O, Rocha EP, Brisse S, Lecuit M. Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity. **Nat Genet.** 2016 Feb 24;48(3):308-13.

Gessain G, Tsai YH, Travier L, Bonazzi M, Grayo S, Cossart P, Charlier-Woerther C, Disson O, Lecuit M. PI3-kinase activation is critical for host barrier permissiveness to *Listeria monocytogenes*. **J Exp Med.** 2015 212(2):165-83

Blériot C, Dupuis T, Jouvion G, Eberl G, Disson O, Lecuit M. Necroptosis of liver-resident macrophages orchestrates type-1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. **Immunity,** 2015 42(1):145-58

Tsai YH, Disson O, Bierne H, Lecuit M. Murinization of internalin extends its receptor repertoire, altering Listeria monocytogenes cell tropism and host responses. **PLOS Pathog.** 2013;9(5):e1003381. doi: 10.1371/journal.ppat.1003381.

Travier L, Guadagnini S, Gouin E, Dufour A, Chenal-Francisque V, Cossart P, Olivo-Marin JC, Ghigo JM, Disson O, Lecuit M. ActA promotes Listeria monocytogenes aggregation, intestinal colonization and carriage. **PLOS Pathog.** 2013 Jan;9(1):e1003131. doi: 10.1371/journal.ppat.1003131.

Nikitas G, Deschamps C, Disson O, Niault T, Cossart P, Lecuit M. Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. **J Exp Med.** 2011 Oct 24;208(11):2263-77.

Disson O, Grayo S, Huillet E, Nikitas G, Langa-Vives F, Dussurget O, Ragon M, Le Monnier A, Babinet C, Cossart P, Lecuit M. Conjugated action of two species-specific invasion proteins for fetoplacental listeriosis. **Nature**. 2008 Oct 23;455(7216):1114-8.

Lecuit M, Vandormael-Pournin S, Lefort J, Huerre M, Gounon P, Dupuy C, Babinet C, Cossart P. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. **Science.** 2001 Jun 1;292(5522):1722-5.

Scientific or technical background required for work programs

Applicants should have a background in either cell/tissue biology, microbiology, imaging and be highly motivated and ambitious. Technical experience in molecular biology and biochemistry is highly recommended. Good communication skills in English are required.

Title of the work program 19:

Use of human iPS cells to study polymorphisms in nicotinic receptor genes

Description of the work program

The targeted differentiation of human induced pluripotent stem cells (hiPS) offers today the unique opportunity to get access to human neurons, a cell type that cannot be sampled in living human patients for disease modelling or drug testing. They can be derived from normal subjects, and more importantly, patients bearing pathological mutations. Another advantage of iPS cells is the possibility to introduce mutations using homologous recombination, employing for example the technology of Zinc Finger Nucleases (ZFNs). There, defined mutations can be introduced into neurons from different donors, to control for the contribution of the individual genes.
We propose to use this paradigm to introduce human polymorphisms predisposing to lung cancer and *nicotine, alcohol and heroin* addiction into iPS cells. These will then be differentiated into the appropriate neuronal subtype, and their properties studied. This human culture system will also be used to screen for compounds specifically interacting with human native nicotinic and *polymorphic* receptors, for drug discovery approaches. This will allow to define a genetic testing system in humans, containing functionally validated SNPs (**s**ingle **n**ucleotide **p**olymorphisms).

a. The objective

Differentiated human neurons will be analysed extensively by calcium imaging and electrophysiology *in vitro* and *in vivo*.

b. The experimental strategy with models used

The different neuronal subtypes will be analysed by calcium imaging and electrophysiology. Functional imaging procedures are well established in U. Maskos' laboratory, and have been used to study the function of the α5-SNP in epithelial cells, as shown in **Figure 2.**

Hiányoznak vagy helytelenek egyes kötelező paraméterek.

Figure 2. Calcium permeability measurements. Concentration-response curves for nicotine-evoked changes in intracellular calcium were measured from primary progenitor tracheal basal cells from α5-/- mice transduced with lentiviral vectors co-expressing either α5D398 (WT) with GFP, α5N398 (SNP) with GFP, or only GFP, preincubated with α-bungarotoxin (10μM).

Electrophysiological characterisation will be carried out as described by the Corringer lab in the patch-clamp configuration. Neurons will be characterized by full dose-response curves for acetylcholine and nicotine, by the kinetics of desensitization and by the permeability of their channel to calcium ion, these two latter features being reported to be altered in the α5SNP context (Kuryatov, Berrettini et al. 2010, George, Lucero et al. 2012, Tammimaki, Herder et al. 2012).

Additionally, human neurons will be transplanted *in utero* as outlined in **Figure 3**, from (Brustle, Maskos et al. 1995, Maskos, Brustle et al. 2001).

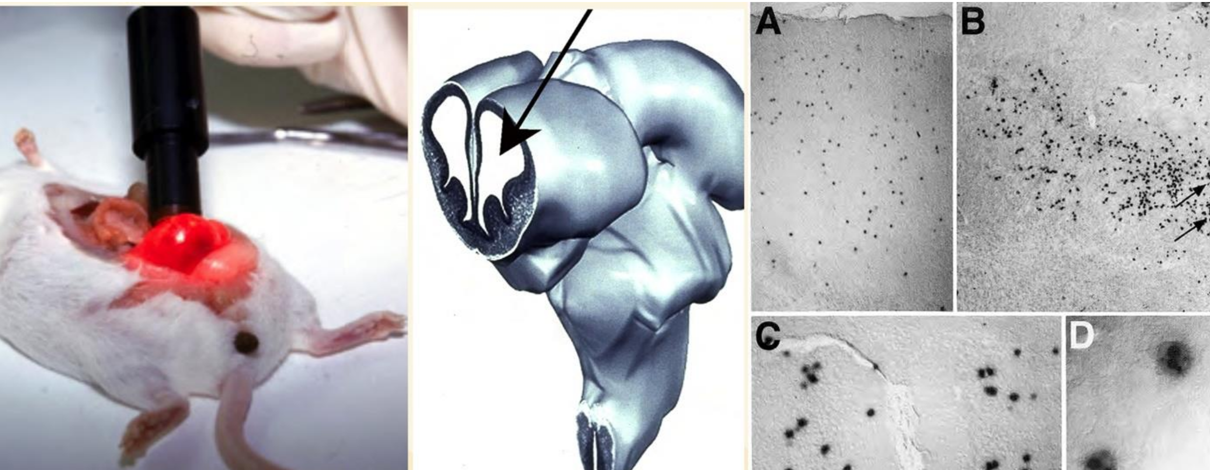

Figure 3: Method used for the analysis of human neurons. *Left*, surgical procedure used to target the ventricle of embryos. *Middle*, Scheme highlighting the ventricle for the grafting of human neurons. *Right*, example of integration of neurons into host parenchyma, A, cortex; B, thalamus; C, putamen and septum; D, high power of neurons detected by species-specific *in situ* hybridization.

Slices from different brain areas will be prepared. Human dopaminergic neurons will be identified by the expression of eGFP after gene transfer with a vector expressing eGFP under the control of a promoter specifically expressed in dopaminergic neurons, the tyrosine hydroxylase promoter.
Patch clamp analysis and calcium imaging in slices will be carried out as described (Frahm, Slimak et al. 2011). The activity of novel compounds can also be analysed in vivo using functional two-photon imaging. This can be adapted to human neurons integrated into the upper layers of cortex. The method is well established in the Maskos laboratory, see **Figure 4**.

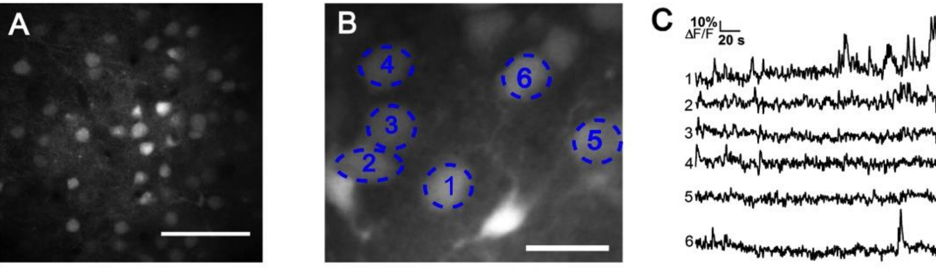

Figure 4. A. *In vivo* two-photon image of layer 2/3 neurons of an adult mouse loaded with OGB-1 AM, 180 µm below the cortical surface. Scale bar 200µm. **B,C.** Spontaneous Ca^{2+} activity recorded in six cells simultaneously. Scale bar, 100µm.

Tutor/supervisor

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

Modeling amyotrophic lateral sclerosis in pure human iPSc-derived motor neurons isolated by a novel FACS double selection technique.
Toli D, Buttigieg D, **Blanchard S**, Lemonnier T, Lamotte d'Incamps B, Bellouze S, Baillat G, **Bohl D**, Haase G.
Neurobiol Dis. 2015 Oct;82:269-80. doi: 10.1016/j.nbd.2015.06.011. Epub 2015 Jun 21.

Modeling neuronal defects associated with a lysosomal disorder using patient-derived induced pluripotent stem cells.
Lemonnier T, **Blanchard S**, Toli D, Roy E, Bigou S, Froissart R, Rouvet I, Vitry S, Heard JM, Bohl D.
Hum Mol Genet. 2011 Sep 15;20(18):3653-66. doi: 10.1093/hmg/ddr285. Epub 2011 Jun 17.

Directed evolution of motor neurons from genetically engineered neural precursors.
Bohl D, Liu S, **Blanchard S**, Hocquemiller M, Haase G, Heard JM.
Stem Cells. 2008 Oct;26(10):2564-75. doi: 10.1634/stemcells.2008-0371. Epub 2008 Jul 17.

Forced expression of the motor neuron determinant HB9 in neural stem cells affects neurogenesis.
Bréjot T, **Blanchard S**, Hocquemiller M, Haase G, Liu S, Nosjean A, Heard JM, **Bohl** D.
Exp Neurol. 2006 Mar;198(1):167-82. Epub 2006 Jan 24.

K Guillem, RB Poorthuis, B Bloem, M Loos, AB Smit, **U Maskos**, S Spijker & H Mansvelder (2011) Nicotinic acetylcholine receptor β2 subunits in the medial prefrontal cortex control attention. *Science* **333**, 888-891.

S Tolu, R Eddine, F Marti, V David, M Graupner, **S Pons**, M Baudonnat, M Husson, **M Besson**, C Reperant, J Zemdegs, C Pagès, YAH Hay, B Lambolez, J Caboche, B Gutkin, AM Gardier, J-P Changeux, P Faure & **U Maskos** (2013) Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. *Mol Psychiatry* **18**, 382-393.

C Morel*, L Fattore*, **S Pons**, A Hay, F Marti, B Lambolez, M De Biasi, M Lathrop, W Fratta, **U Maskos*** & P Faure* (2014) Nicotine consumption is regulated by a human polymorphism in dopamine neurons. *Mol Psychiatry* **19**, 930-936.

Scientific or technical background required for work programs

The student should have knowledge in cell culture, molecular biology, PCR, cloning, and histochemistry.

Brustle, O., U. Maskos and R. D. McKay (1995). "Host-guided migration allows targeted introduction of neurons into the embryonic brain." Neuron **15**(6): 1275-1285.
Frahm, S., M. A. Slimak, L. Ferrarese, J. Santos-Torres, B. Antolin-Fontes, S. Auer, S. Filkin, S. Pons, J. F. Fontaine, V. Tsetlin, U. Maskos and I. Ibanez-Tallon (2011). "Aversion to Nicotine Is Regulated by the Balanced Activity of beta4 and alpha5 Nicotinic Receptor Subunits in the Medial Habenula." Neuron **70**(3): 522-535.
George, A. A., L. M. Lucero, M. I. Damaj, R. J. Lukas, X. Chen and P. Whiteaker (2012). "Function of Human alpha3alpha4alpha5 Nicotinic Acetylcholine Receptors Is Reduced by the alpha5(D398N) Variant." Journal of Biological Chemistry **287**(30): 25151-25162.
Kuryatov, A., W. Berrettini and J. Lindstrom (2010). "Acetylcholine Receptor (AChR) alpha5 Subunit Variant Associated with Risk for Nicotine Dependence and Lung Cancer Reduces (alpha4beta2)2alpha5 AChR Function." Molecular pharmacology **79**(1): 119-125.
Maskos, U., O. Brustle and R. D. McKay (2001). "Long-term survival, migration, and differentiation of neural cells without functional NMDA receptors in vivo." Developmental biology **231**(1): 103-112.
Tammimaki, A., P. Herder, P. Li, C. Esch, J. R. Laughlin, G. Akk and J. A. Stitzel (2012). "Impact of human D398N single nucleotide polymorphism on intracellular calcium response mediated by alpha3beta4alpha5 nicotinic acetylcholine receptors." Neuropharmacology **63**(6): 1002-1011.

Title of the work program 20:

Role of the viral nonstructural 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). We 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). Interestingly, levels of HDL and LDL are altered in patients presenting DHF or DSS, suggesting a possible link between NS1 circulation, lipoprotein dysregulation and vasculopathy.

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. We are currently analyzing the mode of interaction of the NS1 protein with hepatic cells and identifying the signals that may be triggered upon binding to the cell surface or during internalization (Alcon-LePoder *et al.*, 2005). During this training period, the Erasmus student will learn how to characterize the NS1 lipoprotein and associated lipids by biochemical and biophysical methods, study their interaction with target cells by immunofluorescence and assess their potential pro-inflammatory activity by ELISA.

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

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

- **Biophysical and biochemical characterization of lipoproteins**
- **Enzyme-linked immunosorbent assay (ELISA)**
- **Cell culture**
- **Epifluorescent microscopy**

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.

Tutor/supervisor

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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.

Flamand M[1], Megret F, Mathieu M, Lepault J, Rey FA, Deubel V. **Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble hexamer in a glycosylation-dependent fashion.** J Virol. 1999 Jul;73(7):6104-10.

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 21:

New algorithms for the exploration of 3D structure of genomes

Description of the work program

To insure the proper transmission of the genetic information over generations, DNA molecules must be duplicated and segregated faithfully in the two daughter cells. Completion of these processes require a carefully regulated folding of chromosomes. Studying the 3D organization of genomes has become in recent years a hotly investigated field, thanks to technical advances in imaging and genomic techniques.

In the lab, we explore the 3D structure of genomes of many different organisms like bacteria, yeasts or human with the 3C (Chromosome Conformation Capture) technology. This technique involves molecular biology steps and captures DNA fragments that were in close proximity inside the genome and which are then sequenced.

The candidate will develop new algorithms to extract and visualise the most relevant and interesting biological information from these Next Generation Sequencing data.

The candidate will evolve in a young and interdisciplinary team and will benefit for facilities and excellent scientific environment of Institut Pasteur located in the center of Paris, France.

Tutor/supervisor

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

1. Generation and Analysis of Chromosomal Contact Maps of Yeast Species - Cournac A, Marbouty M, Mozziconacci J, Koszul R - Methods Mol Biol 2016

2. The 3D folding of metazoan genomes correlates with the association of similar repetitive elements - Cournac A, Koszul R, Mozziconacci J - Nucleic acids research 2015

3. Spatial reorganization of telomeres in long-lived quiescent cells - Guidi M, Ruault M, Marbouty M, Loïodice I, Cournac A, Billaudeau C, Hocher A, Mozziconacci J, Koszul R, Taddei A - Genome biology 2015

4. Genome-wide replication landscape of Candida glabrata - Descorps-Declère S, Saguez C, Cournac A, Marbouty M, Rolland T, Ma L, Bouchier C, Moszer I, Dujon B, Koszul R, Richard GF - BMC biology 2015

5. Condensin- and Replication-Mediated Bacterial Chromosome Folding and Origin Condensation Revealed by Hi-C and Super-resolution Imaging - Marbouty M, Le Gall A, Cattoni DI, Cournac A, Koh A, Fiche JB, Mozziconacci J, Murray H, Koszul R, Nollmann M - Molecular cell 2015

6. High-quality genome (re)assembly using chromosomal contact data - Marie-Nelly H, Marbouty M, Cournac A, Flot JF, Liti G, Parodi DP, Syan S, Guillén N, Margeot A, Zimmer C, Koszul R - Nature communications 2014

7. Metagenomic chromosome conformation capture (meta3C) unveils the diversity of chromosome organization in microorganisms - Marbouty M, Cournac A, Flot JF, Marie-Nelly H, Mozziconacci J, Koszul R - eLife 2014

8. 3D genome reconstruction from chromosomal contacts - Lesne A, Riposo J, Roger P, Cournac A, Mozziconacci J - Nature methods 2014

Scientific or technical background required for work programs

The candidate must be familiar with python, script languages (bash, awk...) and Unix environment.

Knowledge in genomics will be a plus.

Good practice and writing in English would be appreciable.

Erasmus+

Institut Pasteur Fall 2016

Title of the work program 22:

Differential susceptibilities of European and Mediterranean populations of Aedes albopictus to Zika virus

Description of the work program

Background

The Asian tiger mosquito Aedes albopictus is currently the most invasive species in the world. Over the past 30 years, this mosquito has rapidly spread from the native tropical Southeast Asia across the world and it is currently found in several tropical, subtropical and temperate countries due to its high ecological plasticity. Its dramatic global expansion has increased public health concern due to its vector competence for several arboviruses, including the most prevalent pathogens of humans: chikungunya virus (CHIKV), dengue viruses (DENV) and Zika virus (ZIKV). In collaboration with the Laboratory of Insect Genomics and Biotechnology of the Department of Biology and Biotechnology at the University of Pavia (Italy), we are analysing the genetic structure and differentiation of geographically-distant populations and its effects on vector competence to arboviruses.

Together with the Pavia Lab, we are approaching an important question: how the population structure and differentiation may impact the variation in vector competence for CHIKV, DENV and ZIKV. We demonstrated that the differentiation of Ae. albopictus population may contribute to explain the differential ability to select the Ae. albopictus- adaptive mutation CHIKV E1-226V mutation (Vazeille et al. Sci. Rep 2016).

Work program

Considering that the European populations of Ae. albopictus show a substantial degree of genetic differentiation and may act as a vector of ZIKV creating a risk for local transmission in Europe (Jupille et al. PNTD 2016), it becomes very important to investigate the level of vector competence for ZIKV of different European populations and even Mediterranean populations of Ae. albopictus. This 6-months project will be leveraged to achieve two main tasks:

- (i) Collections of Ae. albopictus from some European countries (Italy, Spain, France) and Mediterranean countries (Morocco, Lebanon, Turkey), and amplification in insectaries to obtain enough mosquitoes for experimental infections in BSL-3.
- (ii) Experimental infections with ZIKV (Asian and West-African genotypes); the classical parameters describing the vector competence will be measured: infection, dissemination and transmission rates.

The selected student from the Pavia lab will receive one-month training on regulation in BSL-3. Then he/she will run experimental infections, dissections, and saliva collections. The Pavia Lab will genotype the mosquito populations and then define their genetic connectedness. This project will allow to improve the collaboration between our two labs, but also to provide the trainee a stimulating multicultural and multi-disciplinary research environment.

Tutor/supervisor

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

Papers on Zika

1. Amraoui F, Atyame-Nten C, Vega-Rúa A, Lourenço-de-Oliveira R, Vazeille M, AB Failloux. 2016. Culex mosquitoes are experimentally unable to transmit Zika virus. Eurosurveillance, Volume 21, Issue 35, 01 September 2016.
2. Fernandes RS, Campos SS, Ferreira-de-Brito A, Miranda RM, Barbosa da Silva KA, Castro MG, Raphael LM, Brasil P, Failloux AB, Bonaldo MC, Lourenço-de- Oliveira R. 2016. Culex quinquefasciatus from Rio de Janeiro Is Not Competent to Transmit the Local Zika Virus. PLoS Negl Trop Dis. 2016 Sep 6;10(9):e0004993.
3. Jupille H, Seixas G, Mousson L, Sousa CA, Failloux AB. 2016. Zika virus, a new threat for Europe? PLoS Neglected Tropical Diseases 10(8):e0004901.
4. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D, Dupont-Rouzeyrol M, Lourenço-de-Oliveira R, Failloux AB. 2016. Differential Susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika Virus. PLoS Neglected Tropical Diseases 10(3):e0004543.

Scientific or technical background required for work programs

Applicants must have basic knowledge in molecular biology, virology and genetics.

Title of the work program 23:

Assembly of the Escherichia coli cell division machinery

Description of the work program

Cell division is a fundamental process in all organisms. In *Escherichia coli,* the formation of the division septum is catalyzed by a multimolecular machinery (divisome) that is made of more than a dozen of essential proteins (called Fts), among which many are intrinsic membrane proteins (1). This complex assembles into a ring-like structure at the future division site to catalyze the cell septation. However, many questions remain unanswered: (i) the precise function of many of these proteins is still unknown; (ii) in *E. coli*, the Fts proteins are recruited to mid-cell in a linear hierarchical order, as shown with green fluorescent protein (GFP) fusion approaches; yet the molecular basis of this ordered recruitment is still unclear. (iii) little is known about the regulation of the assembly and disassembly of the divisome machinery.

In our laboratory, we have used a bacterial two-hybrid screening technology, BACTH (previously developed by the group) to characterize the interaction network among the different Fts proteins (2). We also used BACTH, in combination with genetic studies and cytological techniques (in situ localization of GFP fusions), to explore the molecular determinants of association between the different Fts proteins and to identify - through BACTH library screening - several small integral membrane polypeptides that were shown to be novel components of the divisome (3, 4). We proposed that these small membrane proteins could stabilize the cell division apparatus in stress conditions

More recently, using the same BACTH screening approach, we identified, in lambdoïd prophages (and/or lambda phage), several genes that encode membrane proteins that were shown to interact with components from the *E. coli* cell division machinery.

The objective of the research project will be to further characterize the interactions between these bacteriophages proteins and the *E. coli* Fts proteins. The project will involve various *in vivo* and *in vitro* approaches such as, molecular genetics, bacterial two-hybrid screening, bacteriology, biochemistry, and fluorescence imaging. A first aim will be to decipher the molecular determinant of the interactions between these bacteriophages proteins and the Fts proteins. A second aim will be to assess the physiological implications of these interactions, particularly with regard to the potential control of bacterial cell division by bacteriophage components. A third aspect will be to exploit this knowledge in the design and the *in vivo* screening of small-molecules able to disrupt key interactions between divisomal proteins. Inhibitors of bacterial cell division assembly appear indeed as attractive leads for the development of novel antibiotics (5).

1. **Rowlett VW, Margolin W.** (2015) The bacterial divisome: ready for its close-up. Phil. Trans. R. Soc. B 370: 20150028. http://dx.doi.org/10.1098/rstb.2015.0028

2. **Karimova G., Dautin N., Ladant D.** (2005) Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. *J. Bact.*. **187**:2233-2243

3. **Robichon C, Karimova G, Beckwith J, Ladant D.** (2011) Role of leucine zipper motifs in the association of the Escherichia coli cell division proteins FtsL and FtsB. *J. Bact.* 193:4988-4992

4. **Karimova G, Davi M, Ladant D. (**2012**)** The β-lactam resistance protein Blr, a small membrane polypeptide, is a component of the *Escherichia coli* cell division machinery. *J Bacteriol.* 194 : 5576-5588

5. **den Blaauwen T, Andreu JM, Monasterio O.** (2014) Bacterial cell division proteins as antibiotic targets. Bioorg Chem. 55:27-38

Institut Pasteur Fall 2016

Tutor/supervisor

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

1. **G. Karimova, N. Dautin, and D. Ladant** (2005) Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. *J. Bact.*. **187**:2233-2243
2. **Ouellette SP, Karimova G, Subtil A, Ladant D.** (2012) *Chlamydia* co-opts the rod shape-determining proteins MreB and Pbp2 for cell division. *Mol. Microbiol.* **85**:164-178
3. **Karimova G, Davi M, Ladant D. (**2012**)** The β-lactam resistance protein Blr, a small membrane polypeptide, is a component of the *Escherichia coli* cell division machinery. *J Bacteriol.* 194 : 5576-5588
4. **Ouellette S.P., Rueden K., Gauliard E, Persons L., de Boer P., and D. Ladant** (2014) Analysis of MreB interactors in Chlamydia reveals a RodZ homolog but fails to detect an interaction with MraY. *Front. Microbiol.* **5**:279 doi: 10.3389/fmicb.2014.00279
5. **Gauliard E., S.P. Ouellette, K. Rueden and D. Ladant** (2015) Characterization of interactions between inclusion membrane proteins from Chlamydia trachomatis. Frontiers in Cellular and Infection Microbiology **5**:13.

Scientific or technical background required for work programs

The candidate should have degrees and experimental training in Molecular Biology and Biochemistry, or Biotechnology, Chemistry or related disciplines.

Title of the work program 24:

Towards small-molecule compounds interfering with negative regulation of type I IFN signaling

Description of the work program

Our laboratory has a long standing interest in dissecting molecular mechanisms of Type I interferons (IFN) action. Through the study of cells from rare patients we have recently shown that IFN signaling is finely controlled by the integrated action of two negative regulators, USP18 and ISG15, which are products of IFN-stimulated genes. USP18 acts at the level of the IFN receptor as the *bona fide* negative feedback regulator abrogating JAK/STAT activation. The ubiquitin-like ISG15 contributes to negative regulation by preventing USP18 degradation.

Type I interferons exhibit antiviral and immunomodulatory properties, which have been largely exploited in clinical practice. Yet, some patients are resistant to the beneficial action of administered IFN and in some instances lack of clinical response has been linked to sustained expression of USP18. In this project, we seek to obtain evidence as proof of concept that the USP18-based regulatory loop can be manipulated for therapeutic purposes. The long-term goal will be to identify small-molecule compounds that could enhance IFN therapeutic efficacy by interfering with the function of the negative regulators.
The approach is based on the use of a human fibroblastic cell line 2fTGH that express *gpt* (bacterial HPRT-like enzyme) only in the presence of IFN. These cells survive in HAT plus IFN medium but are killed 6-thioguanine (6TG) plus IFN medium, and were successfully used to select IFN-response mutants lacking JAK/STAT elements (Velazquez et al, Cell 1992; Darnell et al, Science 1994). The student will derive USP18-expressing clones that are resistant to IFN (*ie* survive in 6TG plus IFN) and whose phenotype is reversed following silencing of ectopic USP18. The student will aim at identifying and characterizing the ‘best behaving’ clone to be used for testing efficacy of small molecules. Our collaborators have developed an *in silico* platform for predicting best sites on USP18 and ISG15 for small molecule binding. A large library of compounds is being screened *in silico* to select putative binders. Best hits will be tested in the cell-based assay.

Tutor/supervisor

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

- 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
- 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
- Zhang X, Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer S, Yuan C, Volpi S, Li Z, Sanal O, Mansouri D, Tezcan I, Rice GI, Chen C, Mansouri N, Mahdaviani S, Itan Y, Boisson

B, Okada S, Zeng L, Wang X, Jiang H, Liu W, Han T, Liu D, Ma T, Wang B, Liu M, Liu J, Wang QK, Yalnizoglu D, Radoshevich L, Uzé G, Gros P, Rozenberg F, Zhang S-Y, Jouanguy E, Bustamante J, García-Sastre A, Abel L, Lebon P, Notarangelo L, Boisson-Dupuis S, Crow YJ, Casanova J-L* and Pellegrini S*. 2015. Human intracellular ISG15 prevents IFN-alpha-beta over-amplification and auto-inflammation. *Nature***,** 517:89

- SD Speer, Z LI, S Buta, B Payelle-Brogard, L Qian, F Vigant, E Rubino, TJ Gardner, T Wedeking, M Hermann, J Duehr, O Sanal, I Teczan N Mansouri, P Tabarsi, D Mansouri, V Francois-Newton, CF Daussy, MR Rodriguez, DJ Lenschow, Al Freiberg, D Tortorella, B Lee, A García-Sastre, S Pellegrini* and D Bogunovic*. 2016. ISG15 deficiency and increased viral resistance in humans but not mice. Nature Communic, 7:11496
- MEC Meuwissen, Schot R, Buta S, Oudesluijs G, Tinschert S, Speer SD, LI Z, van Unen L, Heijsman D, Goldmann T, Lequin MH, Kros JM, Stam W, Hermann M, Willemsen R, Brouwer RW, Van IJcken WF, Martin-Fernandez M, de Coo I, Dudink J, de Vries FA, Bertoli Avella A, Prinz M, Crow YJ, Verheijen FW#, Pellegrini S#, Bogunovic D* and Mancini GM*. 2016. Human USP18 deficiency underlies type 1 interferonopathy leading to severe pseudo-TORCH syndrome. *J Exp Med*. 213:1163
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Scientific or technical background required for work programs

The student will be committed and motivated, will have a good background and some technical experience in cellular and molecular biology and be familiar with cell signaling mechanisms. Theoretical knowledge in immunology is highly recommended.

Title of the work program 25:

***Shigella flexneri* invasion blocks exocytosis and endocytosis of its host cell : consequences on tissue integrity and pathogenesis**

Description of the work program

Shigella is an enteroinvasive bacterium that induces bacillary dysentery. The delivery of bacterial effectors inside host cells through a type 3 secretion apparatus (T3SA) allows the bacteria to invade epithelial cells, lyse the membrane of its vacuole, replicate and move into adjacent cells, subverting cellular and immune functions. We recently showed that *Shigella flexneri* invasion induces Golgi fragmentation *in vitro* and *in vivo* and a reorganization of the endocytic compartment, causing a block in secretion and recycling of host cell molecules. At least two bacterial effectors were reported to be involved in host secretion blockage: i) VirA, which acts as Rab1GAP, inactivating the small GTPase Rab1 and ii) IpaJ, which is a cysteine protease that eliminates the N-myristoyl modification from Arf GTPases.
Using a synchronized secretion assay, we showed that invasion of epithelial cells by *S. flexneri* abrogates the secretory trafficking of the adhesion molecule E-cadherin and the cytokine TNFα. Cells infected with either Δ*virA* or Δ*ipaJ* mutants did not recover the trafficking of TNFα to the plasma membrane. However, a Δ*virAipaJ* strain, which lacks both VirA and IpaJ effectors, recovered completely the trafficking of the TNFα reporter, suggesting a synergistic effect of VirA and IpaJ in the blocking of the secretory pathway in host cells. To gain an unbiased overview of how these bacterial effectors affect the secretory trafficking of host cell molecules, we performed stable isotope labeling with amino acids in cell culture (SILAC) to characterize by mass spectrometry the secretome of polarized intestinal epithelial cells infected with WT *S. flexneri* or the *S. flexneri* mutants Δ*virA*, Δ*ipaJ* and Δ*virAipaJ*. On the other hand, we showed that VirA and IpaJ effectors are involved in the blockage of Transferrin (Tf) recycling, as cells infected with Δ*virAipaJ* mutant showed recycling kinetics identical to non-infected control cells. Moreover, endocytosis rate of Tf is decreased in *S. flexneri* infected cells, partially due to the activity of VirA and IpaJ.
These results suggest that *S. flexneri* uses synergic mechanisms to block many intracellular trafficking pathways, affecting their essential role in maintenance of epithelial homeostasis and host defense systems.

The main goal of the internship will be to validate the results obtained by mass spectrometry analysis of the host cell secretome. In addition, the student will perform a functional study on interesting proteins from the analysis to understand the impact of this secretion inhibition induced by *Shigella* on intestinal cell polarity, immunity and infection.

Tutor/supervisor

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

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. 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.
3. Ferrari ML & P Sansonetti. 2016. Encyclopedia of Cell Biology. Cellular Invasion by Bacterial Pathogens. 2016, Pages 784–793

Scientific or technical background required for work programs

The student should have knowledge in cell biology, bacteria, biochemistry and microscopy.

The student will learn and perform cell biology experiments on polarized cell and infections. The analysis of the host cell secretion will be done by SDS-PAGE, western blot and ELISA. Fluorescent microscopy will be used to investigate the effects of *Shigella* infection on intestinal cell polarity and tissue integrity.

Institut Pasteur Fall 2016

Title of the work program 26:

Molecular mechanisms of the assembly of actinobacterial cell division machinery

Description of the work program

How cells grow and divide is at the centre of all forms of life. Our understanding of the underlying processes of this fundamental concept has been greatly advanced in recent decades, however many detailed mechanisms of these highly complex and dynamic processes remain elusive, not least due to technical limitations.
Bacterial cell division is a temporally and spatially regulated process coordinated by a multi-protein complex called the divisome. Assembly of the divisome is initiated by a highly conserved bacterial protein, FtsZ, which polymerizes to form the ring structure that marks the site of cell division. Following ring assembly, FtsZ recruits structural and accessory proteins in an ordered manner to build the functional cell division machinery. The key steps in bacterial cell division have been intensively studied in model organisms such as *Escherichia coli* and *Bacillus subtilis*. However, it is largely unclear how FtsZ orchestrates these different functions in *Corynebacteriales*, an order of Gram-positive rod-like actinobacteria that includes important human pathogens such as *Mycobacterium tuberculosis, Mycobacterium leaprae* and *Corynebacterium diphteriae*. Furthermore, eukaryotic-like Ser/Thr protein kinases are tought to play a crucial role in the phospho-regulation of cell division and growth.

The larger aim of our project is to investigate the molecular mechanisms of divisome assembly in *Corynebacteriales* using an integrative approach employing bacterial genetics, structural biochemistry, proteomics and cryo-electron microscopy. The results of this project are expected to have a significant impact not only on our knowledge of a fundamental physiological process in bacteria, but also on antibacterial drug development, as a better understanding of the cell division machinery in *Corynebacteriales* will likely uncover novel attractive drug targets for specific therapeutic intervention against human pathogens such as *M. tuberculosis*.

Considering the interdisciplinary nature of the project, the precise workplan will be defined in concert with the student depending on the experience, background and specific interests of the candidate. In brief, this could for instance be biochemical and biophysical protein characterization of divisome components; protein kinase assays and functional studies, or morphological characterization of mutant cell strains.

Keywords:
Bacterial cell division, signaling proteins, multi-protein complex, integrative biology

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The Structural Microbiology Unit at the Institut Pasteur has a long-standing interest in the biochemical, biophysical and crystallographic studies of proteins involved in microbial physiology and pathogenesis, with a special focus on structural enzymology [see for instance refs. 1-5] and bacterial signalling proteins such as Ser/Thr protein kinases, phosphatases and two-component systems [6-10].

The student will benefit from a stimulating, dynamic environment and active collaborations in the fields of molecular biophysics, structural proteomics and bacterial genetics.

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

1. Buschiazzo A, Amaya MF, Cremona ML, Frasch AC, Alzari PM (2002) *Mol. Cell* **10**, 757-768.
2. Maté MJ, Ortiz-Lombardia M, Boitel B, Haouz A, Tello D, Susin SA, Penninger J, Kroemer G, Alzari PM (2002) *Nature Struct. Biol.* **9**, 442-446
3. Buschiazzo A, Ugalde JE, Guerin ME, Shepard W, Ugalde RA, Alzari PM (2004) *EMBO J.* **23**, 3196-3205.
4. Giganti D, Bouillon A, Tawk L, Robert F, Martinez M, Crublet E, Weber P, Girard-Blanc C, Petres S, Haouz A, Hernandez JF, Mercereau-Puijalon O, Alzari PM, Barale JC (2014) *Nature Commun*. **5**, 4833.
5. Giganti D, Albesa-Jove D, Urresti S, Rodrigo-Unzueta A, Martinez M, Comino N, Barilone N, Bellinzoni M, Chenal A, Guerin ME, Alzari PM (2015) *Nature Chem. Biol.* **11**, 16-18.
6. Ortiz-Lombardía, M., Pompeo, F., Boitel, B. and Alzari, P.M. (2003) *J. Biol. Chem*. **278**, 13094-13100.
7. Bellinzoni, M., Wehenkel, A., Shepard, W. and Alzari, P.M. (2007) *Structure* **15**, 863-872.
8. Wagner T, Bellinzoni M, Wehenkel A, O'Hare HM, Alzari PM (2011) *Chem. Biol.* **18**, 1011-1020.
9. Mechaly AE, Sassoon N, Betton JM, Alzari PM (2014) *PLoS Biol.* **12**, e1001776.
10. Lisa MN, Gil M, Andre-Leroux G, Barilone N, Duran R, Biondi RM, Alzari PM (2015) *Structure* **23**, 1039-1048

Scientific or technical background required for work programs

The candidate should be studying for a University degree that provides a background in either microbiology, molecular biology, biochemistry and/or biophysics. Previous lab experience would be beneficial.

Institut Pasteur Fall 2016

Title of the work program 27:

Dynamic regulation of chromatin and transcription by HP1 Heterochromatin Proteins

Description of the work program

HP1 proteins are typically known to associate to a histone mark linked to chromatin silencing. But on the other hand, HP1g has also been shown to localize within the body of transcriptionally active genes. We focus on the mechanisms linking HP1g to the regulation of chromatin and transcription. For this purpose we are dissecting the functional links between HP1 proteins and chromatin-associated RNA.

The work program will provide an introduction on epigenetic regulatory mechanisms, with a focus on chromatin associated proteins, and it will be based on experimental approaches and laboratory techniques in the fields of molecular biology and cell biology.

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

Harouz H.*, Rachez C.*, Meijer BM., Marteyn B., Donnadieu F., Cammas F., Muchardt C., Sansonetti P., Arbibe L. (2014) Shigella flexneri targets the HP1γ subcode through the phosphothreonine lyase OspF. EMBO J. 33:2606-22.

Ameyar-Zazoua M, Rachez C, Souidi M, Robin P, Fritsch L, Young R, Morozova N, Fenouil R, Descostes N, Andrau JC, Mathieu J, Hamiche A, Ait-Si-Ali S, Muchardt C, Batsché E, Harel-Bellan A. (2012) Argonaute proteins couple chromatin silencing to alternative splicing. Nat. Struct. Mol. Biol. 19:998-1004.

Scientific or technical background required for work programs

Basic scientific knowledge in the fields of transcription and epigenetic regulation.
Technical background in molecular and cellular biology.

Title of the work program 28:

Role of cellular DNA topology as regulator of HIV-1 replication and latency

Description of the work program(s)

Integration is an essential step of retroviral replication, performed by a viral-encoded enzyme, called integrase (IN). In the case of HIV-1, IN is an efficient anti-viral target, and three catalytic inhibitors are associated to poly-therapy treatments. However, the identification of viral strains resistant to these inhibitors leads the scientific community to look for properties of HIV-1 IN, such as its interaction with cellular partners, that could be used as new antiviral targets.

The selectivity of integration in the infected cell genome is also important for retroviral replication. In the case of lentiviruses, such as HIV-1, integration is favored in active genes enriched in specific histone modifications. This selectivity is governed by several cellular parameters, such as nucleosomes, the LEDGF/p75 protein or the nuclear environment. The sites of integration influence the following step of viral expression or silencing. The molecular parameters responsible for this silencing are still under investigation and their characterization should help to identify new antiviral strategies for patients under poly-therapy treatments.

This Erasmus project is focused on a poorly studied but essential parameter regulating HIV-1 integration and replication: the topology of cellular genomic DNA

DNA topology is a major regulator of gene expression. We have recently obtained evidences that this parameter also regulates IN selectivity and viral transcription. Using complementary *in vitro* and *in cellulo* assays, the Erasmus student will modify the expression and/or the activity of cellular topoisomerases and will study the consequences on retroviral replication, especially at the steps of integration and viral expression. He will also perform topological maps of infected cells and will compare these maps with the distribution of integration sites (this *in silico* comparison will be performed In collaboration with the Bio-informatics Hub of the Pasteur Institute).

Results obtained during this internship should reveal a parameter regulating both HIV-1 integration and expression, that could be used as a new antiviral target.

The host team belongs to the Virology Dpt. of the Pasteur Institute and, more recently, to the "Host-Virus Interactions" laboratory of the Cochin Institute (http://www.institutcochin.fr/departments/3i/team-berlioz-emiliani/organigram_team).

Research projects of this lab. are focused on cellular cofactors involved in the interplay between HIV and the infected cell and on the development of new anti-viral therapies targeting virus-host protein interactions. Due to the status of our team, the selected student will have access to the scientific environments and technological resources proposed by both Pasteur and Cochin Institutes.

Tutor/supervisor

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

Publications in the field of HIV integration

- Botbol, Y., Raghavendra, N.K., Rahman, S. Engelman, A. & **Lavigne, M.** (2008) *Nucleic Acids Research* 36, 1237-46
- Morchikh, M., Naughtin, M., Di Nunzio, F. Xavier, J., Charneau, P., Jacob, Y. & **Lavigne, M.** (2013) *PLOS One,* 8(11):e81217
- Lesbats, P., Botbol, Y., Chevereau, G., Vaillant, C., Calmels, C., Arnéodo, A., Androlea, M. L., **Lavigne, M.** & Parissi, V. (2011) *PLOS Pathogens* 7 (2), e1001280
- Benleulmi, M., Matysiak, J., Lesbats, P., Calmels, C., Henriquez, D., Leon, O., Skalka, A.M., Ruff, M., **Lavigne, M.,** Andreola, ML. Parissi, V. (2015) *Retrovirology,* 12: 13
- Naughtin, M., Haftek-Terreau, Z., Xavier, J., Meyer, S., Silvain, M., Jaszczyszyn, Y., Levy, N., Miele, V. Benleulmi, M.S., Ruff, M., Parissi, V., Vaillant, C. & **Lavigne, M.** (2015) *PLOS One* 10:e0129427
- Pasi M. Mornico, D., Volant, S., Juchet, A., Batisse, J., Bouchier, C., Parissi, V., Ruff, M, Lavery, R. & **Lavigne, M.** (2016) *Nucleic Acids Research,* doi: 10.1093/nar/gkw651

Recent publications of the host laboratory:

- Caillet M, Janvier K, Pelchen-Matthews A, Delcroix-Genête D, Camus G, Marsh M, Berlioz-Torrent C. (2011) *PloS Pathogens*, 7(11):e1002347.
- Janvier K, Pelchen-Matthews A, Renaud JB, Caillet M, Marsh M, Berlioz-Torrent C. (2011) *PloS Pathogens*, 7(2):e1001265.
- Frémont S, Gérard A, Galloux M, Janvier, RE Karess, Berlioz-Torrent C. (2013) *EMBO reports* 14, 364-72.
- Gérard A, Soler N, Ségéral E, Belshan M, Emiliani S. (2013) *Retrovirology*,10:13.
- Gérard A, Ségéral E, Naughtin M, Abdouni A, Charmeteau B, Cheynier R, Rain JC, Emiliani S. (2015) *Cell Host Microbe*, 17, 107-17.

Scientific or technical background required for work programs

We are looking for a student showing a strong interest for Enzymology and Virology and motivated by the use of biophysical and genomic strategies. The student will use standard technics of biochemistry, molecular and cellular biology, and more specific tools (In vitro integration assays, mapping of integration sites in infected cells, *in vitro* and in cells studies of DNA topology, CRISPR-dependent gene silencing, HIV-1 dual color vectors). The selected student will also be enthusiastic, curious, motivated and team-oriented.

Title of the work program 29:

Role of transcription factors in the expression of interleukin 10 in human CD4 T cells

Description of the work program

We are interested in understanding the immunomodulatory effect of type I interferon family (IFNα/β) in the development of effector T helper and regulatory T cells in healthy individuals and patients with multiple sclerosis, an inflammatory neurodegenerative disease of the central nervous system. The project aims at determining mechanisms by which IFN potentiates the expression of the anti-inflammatory cytokine IL-10 and the differentiation of type 1 regulatory-like cells (Tr1-like cells) from naïve CD4 T cells stimulated through the T cell receptor (TCR). We have recently identified transcription factors and STAT family members that regulate the TCR/IFN crosstalk towards IL-10 expression. Mechanistic insights into the action of these factors on *IL-10* regulation will be gained through studies of the TCR and IFN signaling pathways, chromatin immunoprecipitation and RNAi assays in primary CD4 T cells and T cell lines. Insights from this project performed with healthy donors may be translated to multiple sclerosis patients, taking advantage of the translational project that we are developing.
(https://research.pasteur.fr/en/program_project/milieu-interieur-labex/).

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

- Zhang X., Bogunovic D., Payelle-Brogard B., Francois-Newton V., Speer S, Yuan C, Volpi S, Li Z, Sanal O, Mansouri D, Tezcan I, Rice GI, Chen C, Mansouri N, Mahdaviani S, Itan Y, Boisson B, Okada S, Zeng L, Wang X, Jiang H, Liu W, Han T, Liu D, Ma T, Wang B, Liu M, Liu J, Wang QK, Yalnizoglu D, Radoshevich L, Uzé G, Gros P, Rozenberg F, Zhang S-Y, Jouanguy E, Bustamante J, García-Sastre A, Abel L, Lebon P, Notarangelo L, Boisson-Dupuis S, Crow YJ, Casanova J-L and Pellegrini S. 2015. Human intracellular ISG15 prevents IFN-α/β over-amplification and auto-inflammation. *Nature,* 517:89-93
- B. Corre, J. Perrier, M. El Khouri, S. Cerboni, S. Pellegrini and F. Michel. 2013. Type I interferon potentiates T-cell receptor mediated induction of IL-10-producing CD4^{+} T cells. *Eur. J. Immunol.,* 43(10):2730-40.

- Z. Li, M. Gakovic, J. Ragimbeau, M-L Eloranta, L Rönnblom, F Michel and S Pellegrini. 2013. Two rare disease-associated Tyk2 variants are catalytically impaired but signaling competent. *J. Immunol.,* 190(5):2335-44.
- Francois-Newton V., Livingstone M., Payelle-Brogard B., Uzé G., and Pellegrini S. 2012. USP18 establishes the transcriptional and anti-proliferative interferon α/β differential. *Biochem. J.* 446, 509-516.
- Francois-Newton V., de Freitas Almeida G., Payelle-Brogard B., Monneron D., Pichard-Garcia, L. Piehler, J., Pellegrini S., and Uzé G. 2011. USP18-based negative feed-back control is induced by Type I and Type III Interferons and specifically inactivates interferons a response. *PLoS ONE* 6(7):e22200.

Scientific or technical background required for work programs

An experience in transcriptomic studies, regulation of gene expression and T cells would be an advantage.

Title of the work program 30:

Development of a multi-component blood stage vaccine for *Plasmodium falciparum* malaria

Description of the work program

All the clinical symptoms of malaria are attributed to the blood stage of the parasite life cycle. During the blood stage *Plasmodium* merozoites invade and multiply within host RBCs. The invasion of RBCs by malaria parasites is mediated by multiple specific interactions between parasite ligands and host receptors. Antibodies targeting such receptor-ligand interactions can block RBC invasion to protect against malaria. We will try to identify critical receptor-ligand interactions that can be targeted in combination to obtain synergistic inhibition of invasion with high efficiency. Parasite proteins such as PfEBA175, PfPLP2, PfRH5, PfCyRP and PfCLAMP, which play key roles in the invasion process, will be targeted WITH ANTIBODIES individually and in combination of 2-3 proteins for inhibition of invasion with specific antibodies. Recombinant PfEBA175 (region II), PfPLP2 (MAC domain), PfRH5, PfCyRP and PfCLAMP will be produced as recombinant proteins, formulated with potent human compatible adjuvants such as GLA-SE and used to raise antibodies in laboratory animals including mice and rabbits. Sera will be tested for recognition of immunogens by ELISA, recognition of native antigens by immunofluorescence assay (IFA) and inhibition of RBC binding. In addition, antibodies against *P. falciparum* antigens described above will be tested individually and in combination of antibodies for inhibition of parasite growth in growth inhibition assays (GIA) with at least 3 different *P. falciparum* isolates. These studies will be used to identify antibody combinations that yield synergistic growth inhibition with high efficiency. Such combinations will be selected for further development as *P. falciparum* malaria vaccine candidates.

Tutor/supervisor

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

1. **Chitnis CE** et al. 2015. Phase I clinical trial of a recombinant blood stage vaccine candidate for *Plasmodium falciparum* malaria based on MSP1 and EBA175. **PLoS One.** 10(4):e0117820.

2. Dawn A, Singh S, More KR, Siddiqui FA, Pachikara N, Ramdani G, Langsley G and **Chitnis CE**. The central role of cAMP in regulating *Plasmodium falciparum* merozoite invasion of human erythrocytes. 2014. PLoS Pathogens. 10(12):e1004520.

3. Siddiqui FA, Dhawan S, Singh S, Singh B, Gupta P, Pandey A, Mohmmed A, Gaur D, **Chitnis CE**. 2013. A thrombospondin structural repeat containing rhoptry protein from *Plasmodium falciparum* mediates erythrocyte invasion. **Cell. Microbiol.** 15(8):1341-56.

Scientific or technical background required for work programs

Background in biochemistry, microbiology, molecular biology and cell biology will be essential. Experience in parasitology is not necessary but must have an interest in applying modern biology to problems in parasitology.