PROPOSALS FOR FULL PHD AT INSTITUT PASTEUR:

Project n°	Page	Department:	Name of the lab:	Project name:	PhD or Post-doc advisor:	Email address:	Web site address of the lab:
1	3	Mycology	RNA Biology of Fungal Pathogens	Study of the lncRNAs in Cryptococcus neoformans	Guilhem Janbon	janbon@pasteur.fr	https://research.pasteur.fr/en/team/rna-biology-of-fungal-pathogens/
2	5	Developmental & Stem Cell Biology	Genetics and Development of Drosophila	Cell Biology of Notch	Francois Schweisguth	fschweis@pasteur.fr	research.pasteur.fr/fr/team/drosophila-developmental-genetics/
5	7	Structural Biology and Chemistry	Biochemistry of Macromolecular Interactions	Deciphering the translocation process of the adenylate cyclase toxin, CyaA, across target cell membrane	Dr Alexandre Chenal	alexandre.chenal@pasteur.fr	https://research.pasteur.fr/en/team/biochemistry-of-macromolecular-interactions/
6	10	Cell biology and infection	Cell biology of microbial infection	Nuclear activities targeted by intracellular bacteria	Agathe Subtil	asubtil@pasteur.fr	https://research.pasteur.fr/en/team/cellular-biology-of-microbial-infection/
7	12	Immunology	Unit of Cytokine Signaling, INSERM U1221	Immunomodulatory activity of type I interferon in the human T cell response	Frédérique Michel	fmichel@pasteur.fr	http://www.pasteur.fr/en/research/immunology/units-groups/cytokine-signalling
8	14	Institut Pasteur de la Guyane	Laboratoire des Interactions Virus-Hôtes	Viral diversity in birds: characterization and drivers of emergence	Anne LAVERGNE	alavergne@pasteur-cayenne.fr	http://www.pasteur-cayenne.fr/la-recherche/nos-equipes/labo_interactions_virus_hotes/
9	17	Microbiology	Unit of Helicobacter Pathogenesis	"USF transcription factors and the oncogenic response to H. pylori infection".	Eliette Touati	etouati@pasteur.fr	http://www.pasteur.fr/en/research/microbiology/units-groups/helicobacter-pathogenesis
11	20	Developmental & Stem Cell Biology	(Epi)genomics of vertebrate development	Mechanisms and function of the 4D-genome architecture	Francois Spitz	francois.spitz@pasteur.fr	https://research.pasteur.fr/en/team/genomics-and-epigenomics-of-vertebrate-development/
12	22	Developmental & Stem Cell Biology	Heart Morphogenesis	Differential growth of the right and left ventricles in development and disease	Sigolène Meilhac	sigolene.meilhac@pasteur.fr	https://research.pasteur.fr/en/team/heart-morphogenesis/
14	24	Genome and Genetics	Functional Genetics of Infectious Diseases	Molecular evolution and viral adaptability in different host environments	Etienne Simon-Loriere / Anavaj Sakuntabhai	etisl@pasteur.fr, anavaj.sakuntabhai@pasteur.fr	https://research.pasteur.fr/en/team/functional-genetics-of-infectious-diseases/

15	27	Structural Biology and Chemistry	Structural Microbiology	Hunting and studying a hybrid metabolic complex in Actinobacteria	Marco Bellinzoni	marco.bellinzoni@pasteur.fr	https://research.pasteur.fr/en/team/group-marco-bellinzoni/
16	30	Immunology	Lymphocyte Cell Biology Unit	The tumor suppressor Adenomatous polyposis coli as a regulator of anti-tumor immunity	Dr. Vincenzo DI BARTOLO	vincenzo.di-bartolo@pasteur.fr	https://research.pasteur.fr/en/team/lymphocyte-cell-biology/
17	33	Structural biology and chemistry	Pole Proteins	Proteochemometrics approach to the pharmacological modulation of protein-protein interactions	Olivier Sperandio	olivier.sperandio@pasteur.fr	https://research.pasteur.fr/en/team/proteins/
21	36	Developmental and Stem Cell Biology	Unit of Human Developmental Genetics	Understanding cell fate choice during human sex determination	Ken McElreavey	kenme@pasteur.fr	https://research.pasteur.fr/en/team/human-developmental-genetics/
22	40	Virology	Molecular Genetics of RNA Viruses	Exploring the links between hepatitis C virus (HCV) genetic variability and virus-induced metabolic disorders	Dr. Annette Martin	annette.martin@pasteur.fr	https://research.pasteur.fr/fr/team/molecular-genetics-of-rna-viruses/
23	43	Parasites and Insect Vectors	Malaria: Parasites & Hosts	Plasmodium vivax, relapses, genotyping, serology	Ivo Mueller	ivo.mueller@pasteur.fr	https://research.pasteur.fr/en/team/malaria-parasites-and-hosts/
24	46	Cell Biology and Infection	Bioimage Analysis Unit	Machine Learning in computational pathology: application in breast cancer diagnosis	Jean-Christophe Olivo-Marin	jcolivo@pasteur.fr	https://research.pasteur.fr/fr/team/bioimage-analysis/

Project number	1
Title of the PhD or postdoctoral project:	Study of the lncRNAs in Cryptococcus neoformans
Keywords:	RNA biology , Cryptococcus neoformans, lncRNA, RNA-Seq
Department:	Mycology
Name of the lab:	RNA Biology of Fungal Pathogens
Head of the lab:	Guilhem Janbon
PhD or Post-doc advisor:	Guilhem Janbon
Email address:	janbon@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/rna-biology-of-fungal-pathogens/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab, available ONLY for Mexican researchers who already have a working contract in their home country)
Doctoral school affiliation and University	BioSPC Paris Descartes
Research topic	Molecular and cell biology, Microbiology, Mycology, Genetics
Presentation of the laboratory and its research topics:	Presentation of the laboratory and its research topics: The Unit RNA Biology of Fungal Pathogens is a new unit of the Institut Pasteur that will be created in October 2015 in the department of Mycology. It will focus on the study of different aspects of the RNA metabolism of pathogenic fungi. These themes of research include the study of alternative splicing, alternative polyadenylation, alternative transcription start and lncRNA expression in these fungi. The team of Guilhem Janbon is proficient in NGS data analysis and in the molecular biology of the main fungal pathogens. Although most of the research is done using the basidiomycete yeast Cryptococcus neoformans, Aspergillus fumigatus and Candida albicans are also studied.
List your five primary research papers:	1. Jiang, N., Yang, Y., Janbon, G., Pan, J. & Zhu, X. (2012) Identification and functional demonstration of miRNAs in the fungus Cryptococcus neoformans. PLoS One 7, e52734 2. 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). 3. Janbon G., Ormerod K.L., Paulet D., Byrnes III, E.J., Yadav Y., Chatterjee G., Mullapudi N., Hon C.H, Billmyre R.B., Brunel F., Bahn Y.S., Chen W., Chen Y., Chow E.W.L., Coppée J-Y., Floyd-Averette A., Gaillardin C., Gerik K.J., Goldberg J., Gonzalez-Hilarion S., Gujja S., Hamlin J.L., Hsueh Y.P., Ianiri G., Jones S., Kodira C.D., Kozubowski L., Lam W., Marra M., Mesner L.D., Mieczkowski P.A., Moyrand F., Nielsen K., Proux C., Rossignol T., Schein J.E., Sun S., Wollschlaeger C. Wood I.A., Zeng Q., Neuvéglise C., Newlon C.S., Perfect J.R., Lodge, J.K., Idnurm A., Stajich J.E. Kronstad, J.W., Sanyal K. Heitman J., Fraser J.A., Cuomo C.A. & F.S. Dietrich (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) 4. 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. 5. Jung K.W., Yang D.H., Maeng S., Lee K.T., So Y.S., Hong J., Choi J.Y., Byun H.J., Kim H., Bang S., Song M.H., Lee J.W., Kim M.S., Kim S.Y., Ji J.H., Park G., Kwon H., Cha S., Meyers G., Wang L.L., Jang J., Janbon G., Adedoyin G., Kim T., Averette A.K., Heitman J., Cheong E., Lee Y.H., Lee Y.W. & Bahn Y.S. (2015) Systematic functional profiling of transcription factor networks in Cryptococcus neoformans. Nature Com. , 6757 ((Recommended by F1000).
Description of the project:	Cryptococcus neoformans is a basidiomycete yeast responsible for deadly infections in immunocompromised patients. It is globally distributed and causes pneumonia and meningoencephalitis in an estimated 1 million people annually, leading to ~620,000 deaths per year. Diverse studies suggest that the infectious strain is acquired very early in life and can remain for years in a dormant state within alveolar macrophages. As soon as a default in the host immune defenses occurs, the yeasts can multiply and disseminate causing eventually deadly meningoencephitis. C. neoformans cells are able to persist and replicate in the cerebrospinal fluid despite treatment with antifungal agents. The fungal attributes that contribute to adaptation and persistence in the host, immune evasion and altered susceptibility to approved antifungal drugs are currently unknown. In this organism, nearly all the genes are interrupted by small introns which are necessary for gene expression. Our recent analysis of the transcriptome structure revealed also the existence of numerous lncRNAs in C. neoformans var. grubii. These lncRNAs are usually spliced and are mostly antisense of coding genes although their functions are completely unknown. Our preliminary unpublished observations also revealed that their expressions are massively up regulated at 37°C suggesting a possible role of these lncRNAs in virulence. In addition, we demonstrated that one histone deacetylase seems to control le level of expression of at least some lncRNAs. The proposed project aimed to understand the regulation of lncRNAs during infection and their function in virulence. Global analysis (RNA-Seq and ChIP-Seq) will be used to understand the relationship between alteration of the chromatin structure and lncRNAs expression and splicing in response to different environmental cues. The mechanisms of regulation of the expression and maturation of these lncRNAs will be also studied. More specifically, the role of the different C. neoformans histone deactylases (there are 7 of them in the C. neoformans genome) in the regulation of lncRNAs and virulence will be studied. Finally, the potential use of histone deactetylase inhibitors alone or in combination with known antifungal against C. neoformans will be evaluated.
References:	Kwon-Chung K.J., Fraser J.A., Doering T.L., Wang Z., Janbon G., Idnurm A., & Bahn Y.S. (2014) Chapter 22. Cryptococcus neoformans and Cryptococcus gattii, the etiologic agents of cryptococcosis. Casadevall A., Mitchel A., Berman J., Kwon-Chung J., Perfect J., and Heitman J. Cold Spring Harbor Perspect Med. Cold Spring Harbor Laboratory Press. Kung JTY, Colognori D, Lee JT. (2013 Long Noncoding RNAs: Past, Present, and Future. Genetics 193:651-669.
Expected profile of the candidate:	The candidate should be interested in genetics and molecular biology. An interest in bioinformatics would be a plus.

Project number:	2
Title of the PhD or postdoctoral project:	Cell Biology of Notch
Keywords:	Notch, Drosophila, Cell Polarity, Patterning
Department:	Developmental Stem Cell Biology
Name of the lab:	Genetics and Development of Drosophila
Head of the lab:	Francois Schweisguth
PhD or Post-doc advisor:	Francois Schweisguth
Email address:	fschweis@pasteur.fr
Web site address of the lab:	research.pasteur.fr/fr/team/drosophila-developmental-genetics/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University :	CdV
Research topic	Molecular and cell biology
Presentation of the laboratory and its research topics:	We study morphogenesis and address how patterns of cell shape and fate emerge during the development of multicellular organisms. To go beyond observations and decipher the inner logic of morphogenesis, we are using genetics, genome engineering, quantitative live imaging and computation to perturb, measure and (in collaboration) model morphogenesis in fruit flies. Currently, we are studying how epithelia fold (gastrulation), how epithelial cell polarity is developmentally remodelled (asymmetric cell division), how regular patterns of stripes and dots are produced in epithelia (self-organization and Notch dynamics) and how Notch receptor signaling is regulated by polarity and membrane trafficking (cell biology of Notch). For more info, see: research.pasteur.fr/en/team/drosophila-developmental-genetics/
List your five primary research papers:	L. Couturier, N. Vodovar and F. Schweisguth (2012) Endoyctosis by Numb breaks Notch symmetry at cytokinesis. Nature Cell Biology, 14, 131-9 S. Chanet and F. Schweisguth (2012) Regulation of epithelial polarity by the E3 ubiquitin ligase Neuralized and the Bearded inhibitors in Drosophila. Nature Cell Biology, 14, 467-76 L. Couturier, M. Trylinski, K. Mazouni, L. Darnet and F. Schweisguth (2014) A fluorescent tagging approach in Drosophila reveals late endosomal trafficking of Notch and Sanpodo. The Journal of Cell Biology, 3, 351-63 C. Besson, F. Bernard, F. Corson, H. Rouault, E. Reynaud, A. Keder, K. Mazouni and F. Schweisguth (2015) Planar Cell Polarity breaks the symmetry of PAR protein distribution prior to mitosis in Drosophila Sensory Organ Precursor cells. Current Biology, 25, 1104-10 S. Pontier and F. Schweisguth (2015) A Wolbachia-sensitive communication between male and female pupae controls gamete compatibility in Drosophila. Current Biology, 25, 2339-48

Description of the project:	Cell-cell signaling mediated by Notch receptors regulates a wide range of developmental processes in animal species (1). In mammals, Notch controls homeostasis in adult tissues (skin, intestine, blood vessels) and perturbations of Notch activity underlie various adult-onset diseases in humans (T-cell leukemia, Multiple Sclerosis, hypertension, etc...). Thus, understanding the logic of Notch signaling is of general interest. 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 γ-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. 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). We therefore suggest that the relative levels of the receptor and of its ligands at a given membrane domain contribute to the activation/inhibition of Notch and that membrane trafficking is likely to play a key role in regulating these levels. 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. In recent years, we have used genome engineering (including CRISPR-mediated homologous recombination) to develop functional fluorescently tagged receptors (3,4). These fluorescent molecules have allowed us to precisely monitor when and in which cells Notch is activated in the context of Notch-mediated binary fate decisions in the sensory bristle lineage. We propose to now extend this approach to photo-convertible reporters and ask when, where (at the subcellular level) and how Notch is activated. We also propose to use optogenetic approaches (5) to develop reagents allowing us to manipulate when and where Notch is trans-activated and cis-inhibited. In doing so, we will uncover key cell biological mechanisms regulating the in vivo activity of Notch.
References:	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.
Expected profile of the candidate:	Good background and/or strong interest in cell biology, quantitative approaches and microscopy.

Project number	5
Title of the PhD or postdoctoral project:	Deciphering the translocation process of the adenylate cyclase toxin, CyaA, across target cell membrane
Keywords:	protein membrane interaction, protein membrane translocation, lipid bilayer, biophysics, fluorescence, FRET, FTIR, CD, biochemistry
Department:	Structural Biology and Chemistry
Name of the lab:	Biochemistry of Macromolecular Interactions
Head of the lab:	Dr Daniel Ladant
PhD or Post-doc advisor:	Dr Alexandre Chenal
Email address:	alexandre.chenal@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/biochemistry-of-macromolecular-interactions/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Co-direction PhD (6 months at your lab at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	BioSPC
Research topic	Molecular and cell biology, Microbiology, Bio-informatics, Infectious diseases
Presentation of the laboratory and its research topics:	The main objectives of our Research Unit “Biochemistry of Macromolecular Interactions ” is to decipher the molecular basis of action of two bacterial adenylate cyclase toxins that are key virulence factors from Bordetella pertussis (CyaA) and Pseudomonas aeruginosa (ExoY), two important human pathogens. Fundamental knowledge on the original mechanisms of action of CyaA is exploited in translational science for development of innovative therapeutic vaccines, anti-infective strategies, and novel biological screening techniques, such as the bacterial adenylate cyclase two-hybrid system. Our Research Unit has previously made some major contributions in the study of the adenylate cyclase (CyaA) toxin from B. pertussis, the causative agent of whooping cough, particularly in the engineering of CyaA into a potent antigen-delivery vehicle that has recently entered into clinical trials. More recently, we developed a method to produce a monomeric, stable and functional CyaA protein that paves new ways to investigate the molecular processes involved in the intoxication mechanism of CyaA, including its calcium-dependent folding and its translocation process across the plasma membrane of target cells. Our projects are build on the established skills of the team in molecular biology, protein engineering, biochemistry and biophysics of proteins and membranes, and rely extensively on collaborations with numerous groups and facilities from Institut Pasteur as well as from national and international Institutions.
List your five primary research papers:	List of last publications on the topic: - O'Brien DP, Hernandez B, Durand D, Hourdel V, Sotomayor-Pérez AC, Vachette P, Ghomi M, Chamot-Rooke J, Ladant D, Brier S, Chenal A. Structural models of intrinsically disordered and calcium-bound folded states of a protein adapted for secretion. Sci Rep. 2015 Sep 16;5:14223. - Karst JC, Ntsogo Enguéné VY, Cannella SE, Subrini O, Hessel A, Debard S, Ladant D, Chenal A. Calcium, acylation, and molecular confinement favor folding of Bordetella pertussis adenylate cyclase CyaA toxin into a monomeric and cytotoxic form. J Biol Chem. 2014 Oct 31;289(44):30702-16. - Subrini O, Sotomayor-Pérez AC, Hessel A, Spiaczka-Karst J, Selwa E, Sapay N, Veneziano R, Pansieri J, Chopineau J, Ladant D, Chenal A. Characterization of a membrane-active peptide from the Bordetella pertussis CyaA toxin. J Biol Chem.

	2013 Nov 8;288(45):32585-98. - Sotomayor-Pérez AC, Subrini O, Hessel A, Ladant D, Chenal A. Molecular crowding stabilizes both the intrinsically disordered calcium-free state and the folded calcium-bound state of a repeat in toxin (RTX) protein. J Am Chem Soc. 2013 Aug 14;135(32):11929-34. - Veneziano R, Rossi C, Chenal A, Devoisselle JM, Ladant D, Chopineau J. Bordetella pertussis adenylate cyclase toxin translocation across a tethered lipid bilayer. Proc Natl Acad Sci U S A. 2013 Dec 17;110(51):20473-8. - Karst JC, Barker R, Devi U, Swann MJ, Davi M, Roser SJ, Ladant D, Chenal A. Identification of a region that assists membrane insertion and translocation of the catalytic domain of Bordetella pertussis CyaA toxin. J Biol Chem. 2012 Mar 16;287(12):9200-12.
Description of the project:	I. Background The adenylate cyclase toxin (CyaA) plays an important role in the early stages of respiratory tract colonization by B. pertussis, the causative agent of whooping cough. CyaA is a 1706-residue long protein organized in a modular fashion, synthesized as an inactive precursor, pro-CyaA, that is converted into the active toxin upon specific acylation of two lysine residues. One of the main originalities of CyaA stems from its unique mechanism of penetration into eukaryotic cells: a direct translocation of the catalytic domain across the plasma membrane. The molecular mechanism by which CyaA enters into target cells remains, however, largely unknown. Once translocated, ACD binds to the endogenous cytosolic calmodulin and produces supraphysiologic levels of cAMP that in turn alters cellular physiology, leading to cell death. The aim of the PhD project is (i) to decipher the structural mechanism of CyaA membrane insertion and catalytic domain transport across the lipid bilayer (ii) to provide new insights into the CyaA toxin translocation process for biotechnological applications, i.e., to improve CyaA-based antigen delivery vehicle and to contribute to the development of a new generation of pertussis vaccine. The biochemical, biophysical and functional properties of CyaA will be characterized using a combination of standard and cutting-edge methodologies. II- Proposed PhD project II.A. Structure of membrane-inserted CyaA and pro-CyaA toxins The conformational changes of CyaA upon membrane interaction will be characterized by a combination of biophysical techniques (CD, FTIR, ATR-FTIR, fluorescence, FRET) in kinetic and steady-state modes available at Institut Pasteur. The low-resolution structure and oligomerization status of CyaA inserted in the membrane will be further investigated by a combination of electron microscopy (EM, IP), neutron specular reflectometry (ILL, Grenoble) and dual polarization interferometry (DPI, IP). Collectively, these data will be crucial to propose a molecular and kinetic description of the membrane insertion process of both CyaA and pro-CyaA. Moreover, the comparison of these two toxins will allow us to decipher the effect of the acylation on the membrane insertion process, which should be different as CyaA does efficiently translocate ACD into the cytosol while ACD is not transported across membrane within pro-CyaA. II.B. Structure of CyaA upon ACD translocation across lipid bilayers We will describe the impact of the acylation on the successive steps of ACD translocation across lipid bilayers in vitro and, as a future perspective, across the target cell membrane in vivo (erythrocytes, alveolar macrophages and dendritic cells). Two fluorescent assays and cryo-EM will be used to monitor the translocation process. Moreover, our in vitro FRET translocation assay should be easily adapted to eukaryotic cells. Altogether, the proposed studies should provide valuable data on the structure and kinetics of the successive steps of the translocation process. Finally, these studies on the intoxication process will be instrumental (i) for the design of improved CyaA-based antigen delivery vectors and (ii) toward the development of a new, safe and efficient pertussis vaccine.

	III. Concluding remarks on the objectives of the PhD project The PhD project aims to solve several unanswered key questions regarding the molecular mechanism of CyaA intoxication: - the successive steps leading to membrane insertion of CyaA, - the structure and oligomerization status of CyaA inserted into membrane, - the effects of CyaA acylation on the membrane insertion process, i.e., the differences of membrane insertion mechanisms between non-acylated proCyaA and acylated CyaA toxins, - the molecular process of ACD translocation across membranes in vitro and in cellula, - the impact of lipid properties on the successive steps leading to ACD translocation
References:	- Karst JC, Ntsogo Enguéné VY, Cannella SE, Subrini O, Hessel A, Debard S, Ladant D, Chenal A. Calcium, acylation, and molecular confinement favor folding of Bordetella pertussis adenylate cyclase CyaA toxin into a monomeric and cytotoxic form. J Biol Chem. 2014 Oct 31;289(44):30702-16. - Masin J, Osicka R, Bumba L, Sebo P. Bordetella adenylate cyclase toxin: a unique combination of a pore-forming moiety with a cell-invading adenylate cyclase enzyme. Pathog Dis. 2015 Nov;73(8):ftv075. doi: 10.1093/femspd/ftv075. Epub 2015 Sep 20. Review - Sotomayor-Pérez AC, Ladant D, Chenal A. Disorder-to-order transition in the CyaA toxin RTX domain: implications for toxin secretion. Toxins (Basel). 2014 Dec 31;7(1):1-20. doi: 10.3390/toxins7010001. Review. - Ladant D, Ullmann A. Bordatella pertussis adenylate cyclase: a toxin with multiple talents. Trends Microbiol. 1999 Apr;7(4):172-6. Review.
Expected profile of the candidate:	During this 3-year PhD project on the translocation process of the CyaA toxin, the PhD student will be trained and exposed to various environments and methods in molecular biology, biochemistry and biophysics of proteins and protein / membrane interactions. The project will be mainly performed in the Unit but also involves several collaborations and therefore requires a strong motivation, a team-spirited PhD student, capable of taking self-initiatives for the benefit of his/her doctoral project.

Project number	6
Title of the PhD or postdoctoral project:	Nuclear activities targeted by intracellular bacteria
Keywords:	Host-pathogen interactions, chromatin, epigenetic, bacterial effector
Department:	Cell biology and infection
Name of the lab:	Cell biology of microbial infection
Head of the lab:	Agathe Subtil
PhD or Post-doc advisor:	Agathe Subtil
Email address:	asubtil@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/cellular-biology-of-microbial-infection/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University	Complexité du Vivant (ED515)
Research topic	Molecular and cell biology, Microbiology, Infectious diseases
Presentation of the laboratory and its research topics:	Our laboratory studies the interactions between intracellular bacteria and their host cells, with the long term goal of finding novel targets to fight infection, as well as of gaining knowledge on basic cell biology processes. We focus on an intracellular bacterium called Chlamydia. Chlamydiae species pathogenic to humans, mainly Chlamydia trachomatis and Chlamydia pneumoniae, cause a number of diseases, including trachoma, pelvic inflammatory disease and pneumonia. Throughout their cycle in the host cell, chlamydiae remain in a membrane-bound compartment. The work of the laboratory focuses mainly on the functional study of proteins secreted by the bacteria into the host cytoplasm, and on the innate response to infection.
List your five primary research papers:	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 Ball SG, Subtil A, Bhattacharya D, Moustafa A, Weber AP, Gehre L, Colleoni C, Arias MC, Cenci U, Dauvillée D. (2013). Metabolic effectors secreted by bacterial pathogens: essential facilitators of plastid endosymbiosis? Plant Cell. Jan;25(1):7-21 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
Description of	Chlamydia are strict intracellular bacteria. Two species are important pathogens

the project:	of humans: C. trachomatis is the agent of trachoma, and is also the primary cause of sexually transmitted diseases of bacterial origin. C. pneumoniae is responsible for community acquired pneumoniae and might be implicated in the development of atherosclerotic plaques. 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. Our team has identified the first chlamydial protein translocated into the host nucleus during infection [1]. We have recently performed a screen to uncover other chlamydial proteins with nuclear tropism, which raised several candidates. The thesis will focus on the functional study of two of these novel nuclear effectors. Since their primary sequence does not give information on their putative targets we will use proximity assays to identify their sites of action. First we will use 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, to identify specific targets on the host chromatin [2]. Complementary to this approach, we will use BioID to identify proteins that are in close proximity to the nuclear effector [3]. For each of the two effectors of interest, we will obtain one deletion mutant strain using allelic exchange [4]. The behaviour of the mutant strains will be first evaluated in culture cells, and in mouse models if relevant. One can expect that several of the nuclear effectors affect host gene expression, and this will be tested by transcriptome analyses, comparing control cells with cells expressing the bacterial effector protein, as well as cells infected with the wild type and deletion mutant strains. Results will be combined with the interactions mapped using DamID and BioID technologies to obtain a global picture of the mechanisms of action of the two nuclear effectors, and the biological outcome of their activity on infection. This work will lead to the discovery of novel mechanisms by which chlamydial proteins manipulate their host at the genomic level. It could lead to the identification of epigenetic marks of infection, which could have long-term effects after the infection is cleared, either naturally or by antibiotic treatment. Various techniques will be used during this PhD, including molecular biology, fluorescence microscopy, flow cytometry, biochemistry, tissue culture. Proteomics and transcriptomics data will be analysed in collaboration with the dedicated technical platforms on campus.
References:	1. 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. 2. van Steensel B, Henikoff S (2000) Identification of in vivo DNA targets of chromatin proteins using tethered dam methyltransferase. Nat Biotechnol 18: 424-428. 3. 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. 4. Mueller KE, Wolf K, Fields KA (2016) Gene Deletion by Fluorescence-Reported Allelic Exchange Mutagenesis in Chlamydia trachomatis. MBio 7.
Expected profile of the candidate:	The student will be highly motivated, hard working, and with a good background in cell biology.

Project number	7
Title of the PhD or postdoctoral project:	Immunomodulatory activity of type I interferon in the human T cell response
Keywords:	Human, CD4 T cell differentiation, signaling, gene expression, type I interferon
Department:	Immunology
Name of the lab:	Unit of Cytokine Signaling, INSERM U1221
Head of the lab:	Sandra Pellegrini
PhD or Post-doc advisor:	Frédérique Michel
Email address:	fmichel@pasteur.fr
Web site address of the lab:	http://www.pasteur.fr/en/research/immunology/units-groups/cytokine-signalling
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University	ED 394, Physiology, Physiopathology and Therapeutic, UPMC University
Research topic	Molecular and cell biology, Medicine, Immunology
Presentation of the laboratory and its research topics:	Work in the Unit of Cytokine Signaling aims at deciphering molecular mechanisms that govern the biological response to the type I IFN family (IFN/) in non immune cells and in the immune system, in humans. Research topics are focused on the IFN signaling pathway and its regulation, the functional impact of genetic variants associated with immune disorders, and the immunoregulatory activity of IFN in T cell-mediated immune responses. We study primary and established cell models. We also develop translational projects.
List your five primary research papers:	- 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. - S. Dong, B. Corre, E. Foulon, E. Dufour, A. Veillette, O. Acuto and F. Michel. 2006. T cell receptor for antigen induces linker for activation of T cell-dependent activation of a negative signaling complex involving Dok-2, SHIP-1, and Grb-2. J. Exp. Med. 203, 11, 2509-18. - Michel, F., Attal-Bonnefoy, G., Mangino, G., Mise-Omata, S. and Acuto, O. 2001. CD28 as a molecular amplifier extending TCR ligation and signaling capabilities. Immunity, 15 (6), 935-45. - Michel, F., Mangino, G., Attal-Bonnefoy, G., Tuosto, L., Alcover, A., Roumier, A.,

	Olive, D. and Acuto, O. 2000. CD28 utilizes Vav-1 to enhance TCR-proximal signaling and NF-AT activation. J. Immunol.,165: 3820-3829
Description of the project:	Type I IFNs exert a complex immunomodulatory activity, which can result in beneficial and deleterious effects depending on the immune context. The project aims at understanding the immunomodulatory activity of IFN in the development and function of effector T helper and regulatory CD4 T cell subsets in healthy individuals and multiple sclerosis patients. One objective is to determine molecular mechanisms by which IFN potentiates the expression of the anti-inflammatory cytokine IL-10 in human CD4 T cells activated through the T cell receptor (TCR). Using large scale transcriptomic and RNAi approaches, we have identified some transcription factors and STAT family members that control the TCR/IFN crosstalk towards IL-10 expression. Mechanistic insights into these factors will be gained by studying the TCR and IFN signaling pathways and performing chromatin immunoprecipitation and RNAi assays in primary CD4 T cells and T cell lines. Another objective is to investigate how IFN promotes the differentiation of type 1 regulatory-like cells (Tr1-like cells) and to better characterize these cells. Our recent RNA sequencing data will have to be analyzed and validated. Multiplex qPCR will be set up and gene profiling will be determined at the single cell level. Tr1 functional activity will be also investigated. Insights from this project will 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/).
References:	- 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-a/b 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.
Expected profile of the candidate:	Skills in transcriptomic studies, regulation of gene expression, T cell responses and bio-informatics

Project number	8
Title of the PhD or postdoctoral project:	Viral diversity in birds: characterization and drivers of emergence
Keywords:	Viruses, Birds, emergence, Amazonia
Department:	Institut Pasteur de la Guyane
Name of the lab:	Laboratoire des Interactions Virus-Hôtes
Head of the lab:	Vincent LACOSTE
PhD or Post-doc advisor:	Anne LAVERGNE
Email address:	alavergne@pasteur-cayenne.fr
Web site address of the lab:	http://www.pasteur-cayenne.fr/la-recherche/nos-equipes/labo_interactions_virus_hotes/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University	ED587 Ecole doctorale Diversités, Santé et Développement en Amazonie, Université de Guyane
Research topic	Molecular and cell biology, Virology, Genetics, Infectious diseases, Health, environment, society
Presentation of the laboratory and its research topics:	The Laboratoire des Interactions Virus-Hôtes (LIVH) is one of the research laboratories of the Institut Pasteur de la Guyane (IPG). Present in French Guiana since 1940, IPG, is part of the Institut Pasteur International Network (IPIN). The main research topics are focused on tropical infectious diseases. IPG is involved in different research activities in virology (arbovirus, herpesvirus, HIV/AIDS, hantavirus, arenavirus and rabies), parasitology (malaria), immunology (leishmaniosis), entomology and epidemiology. These themes are addressed through translational approaches facilitated by the complementarity of the multidisciplinary teams and to the quality of the technical equipment and their constant improvement. IPG has also major roles in public health, housing analysis laboratories and national reference centres for arboviruses, influenza viruses, hantaviruses and for malaria. To conduct all these activities, IPG possesses distinct technological platforms such as two BioSafety level 3 Laboratories (BSL-3), five BSL-2 labs, a common platform dedicated to molecular biology hosting different equipments for automatic genomic extraction, amplification, quantification and sequencing, an insectary for mosquito breeding and a mouse breeding facility. The IPG is also involved in training activities, welcoming every year students up to PhD level and giving lectures at different University levels (L3, M1 and M2) for the Université des Antilles et de la Guyane (UAG). In addition, IPG has strong links with the major French research institutions and cooperates with institutions in neighboring countries (Suriname, Brazil, …).

	The LIVH is currently composed of 6 scientists (3 staff members (PhD, HDR), 1 post-doc, 2 PhD students), 1 engineer in bio-informatics and 3 lab technicians. The main research activities conducted by the laboratory focus on the role of wild mammalian species on viral dynamics and emergence, co-evolutionary process between viruses and their hosts (herpesviruses, hantavirus, arenavirus) and interactions between viruses and hosts (specifically bats) focusing on genetic and immunological aspects. Recently, LIVH moved to viral genomics using next generation sequencing approaches on different types of hosts: rodents as well as bats. Collaborations for field studies are implemented with local non-governmental organizations dedicated to wildlife studies, and for metagenomic analysis with the Institut Pasteur in Paris and the IPIN.
List your five primary research papers:	de Thoisy B, Bourhy H, Delaval M, Pontier D, Dacheux L, Darcissac E, Donato D, Guidez A, Larrous F, Lavenir R, Salmier A, Lacoste V, Lavergne A. (2016). Bioecological drivers of rabies virus circulation in a neotropical bat community. Plos Neglect Trop D. 10(1):e0004378. Lavergne A, de Thoisy B, Tirera S, Donato D, Bouchier C, Catzeflis F, Lacoste V. (2016). Identification of lymphocytic choriomeningitis mammarenavirus in house mouse (Mus musculus, Rodentia) in French Guiana. Infect Genet Evol. 37, 225-230. Lavergne A, de Thoisy B, Donato D, Guidez A, Matheus S, Catzeflis F, Lacoste V. (2015). Patawa virus, a new arenavirus hosted by forest rodents in French Guiana. EcoHealth. 12, 339-346. de Thoisy B, Matheus S, Catzeflis F, Clément L, Barrioz S, Guidez A, Donato D, Cornu JF, Brunaux O, Guitet S, Lacoste V, Lavergne A. (2014). Maripa Hantavirus in French Guiana: Phylogenetic position and predicted spatial distribution of rodent hosts. Am J Trop Med Hyg. 90, 988-992. de Thoisy B, Lavergne A, Semelin J, Pouliquen JF, Blanchard F, Hansen E, Lacoste V. (2009). Outbreaks of disease possibly due to a natural avian herpesvirus infection in a colony of young Magnificent Frigatebirds (Fregata magnificens) in French Guiana. J Wildl Dis. 45, 802-807.
Description of the project:	In a context of global disease emergence, the “One Health concept”, underlining that “the health of humans, animals and ecosystems are interconnected”, became an innovative approach to deal with sanitary issues. This multidisciplinary approach is expected to provide an integrative view of pathogens’ circulation with the aim to decrease the risk of emergence that may originate at the interface between human populations, wild animals and their various environments (1). Birds are natural reservoirs of numerous viral pathogens of which some, such as avian influenza viruses (e.g. H5N1) (Orthomyxoviridae) or West Nile virus (Flaviviridae), have a major impact on human health. Because of several biological, ecological and behavioral characteristics such as: (a) species richness (around 10,000 species) that make birds the most highly diversified tetrapods, (b) global distribution, (c) migratory behaviors with important distances of migrations and periodic close contacts with resident species, (d) roosting habits in habitats shared with other animals and arthropods, (e) specific innate and adaptive immune systems (2), birds play a major role in the emergence and dissemination of novel viruses and in their transmission to other species as observed, for instance, for the avian influenza A virus H7N9 (2, 3). Due to its equatorial location and coastal mud abundances related to closeness of Amazonia, French Guiana, which is located between Suriname and Brazil, is a unique stop-over place in the World for migrating Nearctic and southern Neotropic birds. Indeed, in French Guiana more than 450 indigenous bird species welcomes, every year, thousands of shorebirds from northern Canada and Alaska (4). For most of these Nearctic migratory species, highly nutritive French Guianan mudflats are their main winter quarters. For others, they are an essential step before continuing their

	migration further South to Argentina, Brazil and Uruguay. In this context, French Guiana can be considered as an area of potential emergence of viruses hosted by birds. The objective of the project is to investigate the circulation of several viruses of public health importance in migratory birds (mainly waders) and in local resident species that may, periodically, share habitats with migratory species and consequently may contribute to the amplification and dissemination of viruses brought during migrations. The first task will be to collect samples of migratory and resident species. Field capture efforts will be performed by the Institut Pasteur team with the support of naturalists and NGOs networks on the main coast for waders, with a special attention to populations of resident marine bird species (seagulls, sterns), and in all coastal habitats (beaches, mangroves, littoral forests, marsh and savanna open areas) where migrants and resident species can temporarily get in close contacts. Bird populations will be monitored during a two-years period (during migratory seasons). Blood samples, tracheal and cloacal swabs will be collected, as well as ecto-parasites, if present. The second task will be to monitor the circulation of a selected set of viruses in bird populations. Viruses (influenza viruses, arboviruses and coronaviruses) will be evidenced using standardized methods: serology, PCR, RT-PCR, qPCR. Together with classical approaches, metagenomic analyses of viral diversity will be performed. This will not only allow to characterize novel viruses but also to propose an original model: the virus community as a proxy of migration patterns (diet, period, stress, inter-species contacts) and ultimately emergence risks. Technical and technologic skills (viral diagnosis, next generation sequencing approach, metagenomic analyses) of the lab welcoming the project will make possible, for the first time, investigations on the role and importance of those fascinating virus spreaders on emerging risks in Northern Amazonia.
References:	(1). Rabozzi G, Bonizzi L, Crespi E, Somaruga C, Sokooti M, Tabibi R, Vellere F, Brambilla G, Colosio C. Emerging zoonoses: the "one health approach". Saf Health Work. 2012; 3:77-83. (2). Chan JF, To KK, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 2013; 21(10). (3). Chan JF, To KK, Chen H, Yuen KY. Cross-species transmission and emergence of novel viruses from birds. Curr Opin Virol. 2015; 10:63-9. (4). Hansen-Chaffard, E. 2000. Peuplement des oiseaux d'eau du littoral guyanais cas particulier des limicoles. Ecole pratique des hautes études, sciences de la vie et de la terre.103p.
Expected profile of the candidate:	- The candidate should have experiences in molecular biology in virology (or parasitology) and knowledge on phylogenetic analysis. - The candidate is expected to play an active and collaborative role in the lab, be self motivated, and develop and run independent experiments. - Previous experiences with birds (or mammals) ecology, population dynamics, capture, sampling, will be appreciated. - Work language in the lab: English and French.

Project number	9
Title of the PhD or postdoctoral project:	“USF transcription factors and the oncogenic response to H. pylori infection”.
Keywords:	Host-pathogen interaction, H. pylori, gene regulation, DNA repair, oncogenesis, gastric cancer
Department:	Microbiology
Name of the lab:	Unit of Helicobacter Pathogenesis
Head of the lab:	Hilde De Reuse
PhD or Post-doc advisor:	Eliette Touati
Email address:	etouati@pasteur.fr
Web site address of the lab:	http://www.pasteur.fr/en/research/microbiology/units-groups/helicobacter-pathogenesis
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Co-direction PhD (6 months at your lab at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	Doctoral School BioSPC University Paris Diderot and University Paris Descartes
Research topic	Molecular and cell biology, Microbiology, Infectious diseases
Presentation of the laboratory and its research topics:	In our unit we study the pathogenesis of Helicobacter pylori infection, a bacterial pathogen that colonizes specifically the human stomach of about half of the human population worldwide. Infection by H. pylori is chronic and can evolve from gastritis to severe pathologies such as gastric cancer. We develop complementary approaches to analyse the bacterial physiology of H. pylori and the mechanisms caused by its interaction with the host that are responsible for its pathogenicity. One part of the projects aims at understanding what makes H. pylori such a successful and persistent pathogen in an hostile niche, the acid stomach. This includes a study of the transport, trafficking and sensing of Nickel which is an essential virulence determinant for H. pylori. The analysis of RNA-mediated regulation in H. pylori is also investigated. The second part of the topics is developed by E. Touati ‘s group on “Infection, Genotoxicity and Cancer”. The studies develop by this group aim to characterize molecular events at the origin of the genotoxic activity of H. pylori infection and its oncogenic consequences. We develop two main projects focused on the study of pleiotropic transcriptional regulators, Upstream Stimulating factors (USF) and their involvement in the host response to the infection. The USF factors have been previously demonstrated as stress sensors and would be associated with a tumor suppressive activity. They regulate the expression of genes involved in essential cellular functions including immune response, cell proliferation and maintenance of

	genome stability. In order to identify host factors that play an important role in the pathogenicity associated to H. pylori infection, we also investigate the consequences of H. pylori at mitochondria of which dysfunctions are associated with several human diseases including cancer. Our work is specially focused on mitochondrial genome and mechanisms related to maintenance of its integrity during the infection. Finally, a translational approach is conducted, that aims at identifying biomarkers for an early detection of gastric cancer.
List your five primary research papers:	• Touati E, Michel V, Thiberge JM, Wuscher N, Huerre M and Labigne A (2003) Chronic Helicobacter pylori infection induce gastric mutations in mice. Gastroenterology, 124, 1408-1419. • Vivas JR, Regnault B, Michel V, Bussière FI, Avé P, Huerre M, Labigne A, D'Elios MM and Touati E (2008) Interferon g-signature transcripts profiling and IL-23 upregulation in response to Helicobacter pylori infection. International J. of Immunopathology and Pharmacology, 21 : 515-526. • Machado AM*, Figueiredo C*, Touati E, Máximo V, Sousa S, Michel V, Carneiro F, Nielsen FC, Seruca R, and Rasmussen LJ (2009) Helicobacter pylori infection influences genetic stability of nuclear and mitochondrial DNA. Clinical Cancer Research, 15 : 2995-3002. • Bussière FI, Michel V, Mémet S, Avé P, Vivas JR, Huerre M and Touati E (2010) H. pylori-induced promoter hypermethylation downregulates USF1 and USF2 transcription factor gene expression. Cellular Microbiology, 12 : 1124-1133. • Fernandes J, Michel V, Carmolinga-Ponce M, Gomez A, Maldonada C, De Reuse H, Torres J, Touati E. (2014) Circulating mitochondrial DNA level as a potential non-invasive biomarker to the early detection of gastric cancer. Cancer Epidemiology, Biomarkers and Prevention, 23 : 2430-2438.
Description of the project:	Helicobacter pylori is a gastric pathogen that infects chronically about 50% of the human population worldwide. It induces gastric inflammation that can evolve to severe pathologies as peptic ulcers (10% of the infected population) and gastric cancer (1 to 3%). Up to now, H. pylori is the only bacteria associated with cancer. We explore the events at the origin of the relation between this bacterium and gastric cancer development. We previously demonstrated a mutagenic effect in H. pylori chronically-infected mice associated with gastric inflammation and an impairment of DNA mismatch and DNA base excision repair systems. H. pylori infection is also a source of epigenetic alterations in gastric epithelial cells. Both genetic instabilities and epigenetic mechanisms are known to occur at the initial steps of the carcinogenic process and are proposed to play an important role in H. pylori associated gastric carcinogenesis. Our previous studies showed that H. pylori induces DNA methylation in the promoter region of the Upstream Stimulating Factors USF1 and USF2, leading to the inhibition of their transcription. USF1 and USF2 are pleiotropic transcription factors and key regulators of genes related to stress conditions, cell proliferation, immune response and DNA damage and repair response. They act as homo- or heterodimers. USF1 and USF2 interact with specific E-boxes DNA binding sequences in promoter regions of their target genes. They have been proposed as tumor suppressor genes. Their role in the host response during H. pylori infection and their impact in the associated gastric carcinogenesis remain to be determined. We speculate that by depleting USF1 and USF2, H. pylori would consequently deregulate USF1/USF2-dependent cellular functions thus resulting in the promotion of gastric carcinogenesis. The proposed project of this thesis aims at investigating the consequences of the H. pylori-mediated USF1 and USF2 deregulation on the host response with a special focus on genes related to oncogenesis and associated regulatory pathways, using

	global gene expression profiling. The mechanisms of regulation of the identified genes will be investigated by several molecular and cellular approaches including chromatin immunoprecipitation (ChIP) methods. In order to analyse the consequences of USF depletion on the gastric pathogenicity associated to H. pylori infection, a mouse model will also be used. The second part of the project will compare the deregulation of the USF factors expression by various H. pylori clinical isolates from gastritis and gastric cancer patients and will focus on the identification of H. pylori factors responsible for the deregulation of USF1 and USF2 and their target genes. This includes characterization, by biochemical and genetic approaches, of H. pylori candidates based on their ability to deregulate USF1 and USF2 genes expression in gastric epithelial cells in vitro. In conclusion, the proposed project will combine different complementary approaches that will allow further insights in the role of these pleiotropic transcription factors and their involvement in mechanisms leading to severe clinical outcome of H. pylori infection. It will also lead to the characterization of new H. pylori virulence factors with potential oncogenic properties.
References:	- Bussière FI, et al (2010) H. pylori-induced promoter hypermethylation downregulates USF1 and USF2 transcription factor gene expression. Cellular Microbiology, 12 : 1124-1133. - E.Touati (2010) When bacteria are mutagenic and carcinogenic: lessons from H. pylori. Mutation Research. 703: 66-70. - Bouafia et al, (2014) p53 requires the stress sensor USF1 to direct appropriate cell fate decision, PLOSGenetics, 10; e1004309 -Hardbower at al, (2014), At the bench: Helicobacter pylori, dysregulated host responses, DNA damage and gastric cancer. J of Leukocyte Biology, 96, 201-212
Expected profile of the candidate:	The candidate should have solid knowledge on Microbiology, Host-pathogens interaction, Molecular and Cellular Biology. She/he should be able to work with the mouse model.

Project number	11
Title of the PhD project:	Mechanisms and function of the 4D-genome architecture
Keywords:	Genome organisation; Chromatin; Gene expression; Genomic rearrangements and disease; evolution
Department	DEPARTMENT OF DEVELOPMENTAL & STEM CELL BIOLOGY
Name of the lab:	(Epi)genomics of vertebrate development
Head of the lab:	Francois Spitz
PhD or Post-doc advisor:	Francois Spitz
Email address:	francois.spitz@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/genomics-and-epigenomics-of-vertebrate-development/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Co-direction PhD (6 months at your lab at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University : Paris 6 - Complexité du vivant	
Research topic : Molecular and cell biology, Bio-informatics, Genetics	
Presentation of the laboratory and its research topics:	Our aim is to understand how the structural organization of the genome contributes and influences genomic functions and notably gene expression. We are particularly interested into the role of the 3D architecture of the genome in the regulation of developmental genes by distant cis-acting elements. We use and develop novel in vivo genomic and chromatin engineering strategies as well as functional genomics approaches to investigate and uncover the molecular determinants that translate a linear sequence into specific 3D domains and a highly dispersed regulatory information into specific and robust gene expression programs. We are as well studying how changes in these mechanisms and genome structure contribute to animal evolution and human pathologies.
List your five primary research papers:	1) Tsujimura T, Klein FA, Langenfeld K, Glaser J, Huber W, Spitz F. A discrete transition zone organizes the topological and regulatory autonomy of the adjacent Tfap2c and Bmp7 genes. 2015. PLOS Genetics 11:e1004897. doi: 10.1371/journal.pgen.1004897 2) Uslu VV, Petretich M, Ruf S, Langenfeld K, Fonseca N, Marioni J, Spitz F. 2014. Long-range enhancers regulating Myc expression are required for normal facial morphogenesis. Nature Genetics 46: 753–758. 3) Symmons O, Uslu VV, Tsujimura T, Ruf S, Nassari S, Schwarzer W, Ettwiller L, Spitz F. 2014. Functional and topological characteristics of mammalian regulatory domains. Genome Res. 24: 390–400. 4) Marinić M, Aktas T, Ruf S, Spitz F. 2013. An Integrated Holo-Enhancer Unit Defines Tissue and Gene Specificity of the Fgf8 Regulatory Landscape. Dev Cell 24: 530–542. 5) Ruf S, Symmons O, Uslu VV, Dolle D, Hot C, Ettwiller L, Spitz F. 2011. Large-scale analysis of the regulatory architecture of the mouse genome with a transposon-associated sensor. Nat Genet 43: 379–386.
Description of the project:	In vertebrates, gene expression is controlled by sets of cis-acting elements that can lie several hundreds kilobases away from their associated promoters. Because of the distances involved, the activity of these elements is defined not only by their intrinsic

	regulatory potential, but also by their ability to transfer it to their target genes (1). Recent works have shown that the subdivision of the genome in different structural entities, called "topologically-associating domains" TADs, plays an important role in regulating long-distance regulatory communications (2). TADs define limits to the range of action of enhancers, but also lead to an increase of contact frequency between their sequences, enabling therefore effective long-distance influences. Yet, despite recent progresses, the different factors that define TADs domains, and in particular organize their internal folding so as to regulate specificity and efficiency of enhancer-promoter communications remain unknown. We have recently characterized several enhancers, located several hundreds of kilobases away key developmental genes. Genetic engineering in mice showed that these enhancers are essential for gene expression in specific organs and tissues, as their deletion or impaired communication with their target genes leads to embryonic development and hematopoiesis defects ((3) and unpublished data). We aim to characterize the genomic parameters that regulate the communication between these distant elements and their target genes, as well as their influence on the specificity and the robustness of the gene regulatory programs that control embryonic development. For this purpose, we use a combination of cutting edge experimental genetic and genomic approaches to re-engineer the mouse genome (eg. GROMIT (4,5); CRISPR/Cas9) and analyze its conformation and chromatin organisation (by genomics : 4C/Hi-C; ChIP-Seq; ATAC-Seq; and microscopy: 3D-FISH). We will in particular take advantage of the large collection of chromosomal rearrangements we have established notably around the Myc (3) and Shh loci. The goal of the project is to characterize how these rearrangements alter the overall 3D organisation of the locus, the dynamics interactions between genes and enhancers at those loci, and ultimately impact how these genes are expressed in vivo. An important part of the project - understanding how a linear molecule is folded in 3D in a specific manner - will be done in collaboration with physicists at Pasteur and MIT. Noteworthy, chromosomal rearrangements at the loci we study are associated in humans with developmental malformations and hematopoietic malignancies. Our research program will therefore led to a better understanding of their molecular aetiologies.
References:	1. F. Spitz, Gene regulation at a distance: From remote enhancers to 3D regulatory ensembles. Seminars in cell & developmental biology. 57, 57–67 (2016). 2. J. Dekker, L. Mirny, The 3D Genome as Moderator of Chromosomal Communication. Cell. 164, 1110–1121 (2016). 3. V. V. Uslu et al., Long-range enhancers regulating Myc expression are required for normal facial morphogenesis. Nat Genet. 46, 753–758 (2014). 4. O. Symmons et al., Functional and topological characteristics of mammalian regulatory domains. Genome Res. 24, 390–400 (2014). 5. S. Ruf et al., Large-scale analysis of the regulatory architecture of the mouse genome with a transposon-associated sensor. Nat Genet. 43, 379–386 (2011).
Expected profile of the candidate:	The project(s) developed in the laboratory combine cutting edge functional molecular genetics and genomic technologies in animal models with advanced computation analyses. Computational analyses, imaging and modelling are an integral and growing part of the approaches used and developed in the lab. We are therefore welcoming applicants with either a strong background in experimental biology and genomics, or in computer science/bio-informatics and bio/physics, who express a strong interest to work at the interface of those domains, in a dynamic, international and interdisciplinary environment.

Project number	12
Title of the PhD or postdoctoral project:	Differential growth of the right and left ventricles in development and disease
Keywords:	heart morphogenesis, mouse genetics, congenital heart defects, tissue growth
Department:	Developmental & Stem Cell Biology
Name of the lab:	Heart Morphogenesis
Head of the lab:	Sigolène Meilhac
PhD or Post-doc advisor:	Sigolène Meilhac
Email address:	sigolene.meilhac@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/heart-morphogenesis/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University : ED515 “Life Science Complexity”, University Paris 6	
Research topic : Molecular and cell biology, Genetics	
Presentation of the laboratory and its research topics:	The acquisition of a specific shape is key for organ function. The group of Heart Morphogenesis studies how cells are coordinated at the level of the tissue and how their local behaviour generates global changes of organ shape. We address these questions in the context of heart development, which provides a striking model of morphogenesis in 3D. We use a combination of approaches to address these questions, including genetics, transcriptomics, embryology, primary cultures of cardiac cells, 3D imaging and computer modelling. We have previously characterized the lineages and behaviour of cardiac muscle cells during heart morphogenesis [1, 2]. We have also developed interdisciplinary tools for the quantification of tissue anisotropy in 3D and revealed that myocardial cells coordinate locally their orientation of division during cardiac chamber expansion [3, 4]. Recently, we have studied the atypical cadherin Fat4, a cell adhesion protein, which was initially discovered in the fly as a major regulator of organ size. However, how the Fat pathway is connected to the Hippo pathway in mammals remained poorly understood. We have shown that Fat4 is required to restrict heart growth at birth, by modulating the nuclear translocation of the effector of the Hippo pathway Yap1, in a non-canonical way [5]. In addition to investigating the mechanism of heart growth, we are interested in the looping of the heart tube in the early embryo, which provides an example of how left-right patterning is sensed by cells to impact on morphogenesis. Our work in the mouse is relevant to congenital heart defects and heart repair in humans. The laboratory is affiliated to both the Department of Developmental Biology of the Institut Pasteur as well as the Institut Imagine, within the Hospital Necker-Enfants Malades, in which the national reference centre for congenital heart defects is located.
List your five primary research papers:	1- Oriented clonal cell growth in the developing mouse myocardium underlies cardiac morphogenesis, S. Meilhac, M. Esner, M. Kerszberg, J. Moss and M. Buckingham, The Journal of Cell Biology 2004, 164(1) : 97-109. 2- 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.

	3- 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. 4- 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. 5- 2015 Patent WO/2015/121323 : Treatment of cardiac diseases with modulators of the Hippo pathway
Description of the project:	The mammalian heart has four cardiac chambers, two atria and two ventricles. The right and left ventricles have distinct shapes and sizes, which is in keeping with their distinct function of driving either the systemic or pulmonary circulation of the blood. These differences are already detectable in the embryonic heart. Previous work in the lab has shown for example that growth of the cardiac muscle is oriented specifically in the embryonic right and left ventricle (Meilhac et al., 2004). However, how the specific morphogenesis of cardiac chambers is regulated has remained poorly understood. In pathological conditions, such as transposition of the great arteries, when the left ventricle is connected to the pulmonary artery instead of the aorta, ventricular size is modified. This has dramatic consequences after repair of the connections between the great arteries and the ventricles, as the left ventricle can no longer sustain the high pressure of the systemic circulation. Surgeons have to retrain the left ventricle before repair of such congenital heart defect (Ohye et al., 2015). It is thus of clinical relevance to control the specific growth of cardiac chambers. The PhD project will address the mechanism of the differential growth of the ventricles. Which growth factors are specific to one ventricle ? Can such factors be manipulated to control the specific growth of a ventricle ? The first aim is to characterise the growth of the left and right ventricles during development. Molecular markers will be identified by transcriptomics. Quantification of chamber shape will be performed by High Resolution Episcopic Microscopy (Mohun at al., 2012) and that of myofibre architecture by Magnetic Resonance Imaging (Sosnovik et al., 2014). The second aim is to analyse how the growth of the ventricles is affected in a pathological model of transposition of the great arteries, using the same approaches. Based on the transcriptomic observations in the control and pathological conditions, one most promising growth factor will be selected and, as a third aim, its role in the specific growth of a ventricle will be tested in vivo. The Crispr-Cas9 technology will be used to generate a novel mouse model. The project is expected to provide novel insight into the mechanisms of heart growth and congenital heart defects.
References:	Meilhac S., M. Esner, M. Kerszberg, J. Moss and M. Buckingham, The Journal of Cell Biology 2004, 164(1) : 97-109, Oriented clonal cell growth in the developing mouse myocardium underlies cardiac morphogenesis. Mohun TJ, Weninger WJ., Cold Spring Harb Protoc. 2012 2012(6):641-6, Episcopic three-dimensional imaging of embryos. Ohye RG, Si MS, Bove EL, Hirsch-Romano JC, Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu. 2015;18(1):40-2, Left ventricular retraining: theory and practice. Sosnovik DE, Mekkaoui C, Huang S, Chen HH, Dai G, Stoeck CT, Ngoy S, Guan J, Wang R, Kostis WJ, Jackowski MP, Wedeen VJ, Kozerke S, Liao R., Circulation 2014 129(17):1731-41, Microstructural impact of ischemia and bone marrow-derived cell therapy revealed with diffusion tensor magnetic resonance imaging tractography of the heart in vivo.
Expected profile of the candidate:	A strong interest in developmental biology is required, as well as previous lab experience in molecular or cellular biology. You work with rigour and creativity and enjoy team work.

Project number	14
Title of the PhD or postdoctoral project:	Molecular evolution and viral adaptability in different host environments
Keywords:	virus, evolution, human genetics
Department:	Genome and Genetics
Name of the lab:	Functional Genetics of Infectious Diseases
Head of the lab:	Anavaj Sakuntabhai
PhD or Post-doc advisor:	Etienne Simon-Loriere / Anavaj Sakuntabhai (pending HDR for ESL)
Email address:	etisl@pasteur.fr, anavaj.sakuntabhai@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/functional-genetics-of-infectious-diseases/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	ED BioSPC University Paris Descartes
Research topic	Molecular and cell biology, Virology, Genetics, Infectious diseases
Presentation of the laboratory and its research topics:	The GFMI unit is a multidisciplinary laboratory that includes human geneticists, immunologists, epidemiologists and virologists. We study the basis of human genetic susceptibility to major human pathogens, with a focus on two mosquito-borne infections (malaria and dengue) that impose a heavy public health burden in tropical and sub-tropical regions. We aim to identify genes governing infection outcome and transmissibility, within two important contexts: - That of the pathogen exploitation of the host and maximization of onward transmission; - The environmental context, placing emphasis upon the exogenous factors that impact upon the within-host dynamics of the pathogen and thus influence the outcome of infection.
List your five primary research papers:	Simon-Loriere E, Lin RJ, Kalayanarooj SM, Chuansumrit A, Casademont I, Lin SY, Yu HP, Lert-Itthiporn W, Chaiyaratana W, Tangthawornchaikul N, Tangnararatchakit K, Vasanawathana S, Chang BL, Suriyaphol P, Yoksan S, Malasit P, Despres P, Paul R, Lin YL, Sakuntabhai A. (2015) High Anti-Dengue Virus Activity of the OAS Gene Family Is Associated With Increased Severity of Dengue. J Infect Dis. Dec 15;212(12):2011-20. Simon-Loriere E, Faye O, Faye O, Koivogui L, Magassouba N, Keita S, Thiberge JM, Diancourt L, Bouchier C, Vandenbogaert M, Caro V, Fall G, Buchmann JP, Matranga CB, Sabeti PC, Manuguerra JC, Holmes EC, Sall AA. (2015) Distinct lineages of Ebola virus in Guinea during the 2014 West African epidemic. Nature. Aug 6;524(7563):102-4. Grange L, Simon-Loriere E, Sakuntabhai A, Gresh L, Paul R*, Harris E*. (2014) Epidemiological risk factors associated with high global frequency of inapparent

	dengue virus infections. Front Immunol. Jun 11;5:280. Simon-Loriere E & Holmes EC. Gene duplication is infrequent in the recent evolutionary history of RNA viruses. Mol Biol Evol. 2013 Jun;30(6):1263-9. Pagán I, Holmes EC, Simon-Loriere E. Level of Gene Expression is a Major Determinant of Protein Evolution in the Viral Order Mononegavirales. J Virol. 2012 May;86(9):5253-63.
Description of the project:	Emerging and re-emerging infectious diseases are major threats to human and veterinary public health. They remain among the leading causes of death and disability worldwide and represent a significant burden on global economies. Most importantly, there is a wide variation in both animal and human risk and outcome of infection, generally encompassing asymptomatic, to more severe and sometimes lethal cases. Genetic epidemiology provides solid evidence that genetic variation in human populations contributes to susceptibility to infectious disease. This project aims at exploring the question of human differences of susceptibility to infection and severe disease from a novel virological and evolutionary perspective. More specifically, the aim of this project is to better understand how the host environment may influence the evolutionary trajectories, composition and properties of a viral population, notably with respect to pathogenicity and transmissibility. Dengue virus (DENV) is a perfect example of a pathogen associated with varying degrees of clinical severity, and as such, a highly representative model for this project. Infection with DENV results in a spectrum of clinical outcomes, ranging from self-limiting, uncomplicated dengue fever to the more severe dengue hemorrhagic fever or shock syndrome. In addition, a significant although variable fraction of DENV infections are pauci- or asymptomatic, but play a major role in the continued circulation of dengue viruses. Genetic factors have been shown to influence the risk of severe dengue disease (Rodenhuis-Zybert et al., 2010), and this translates into strong disparities in individual responses to infection, but also at the scale of human populations. For example, Cuba was the stage of several dengue epidemics, during which the proportion of severe cases observed in populations of African origin was significantly reduced compared to populations of European or Asian ancestry (Guzman and Kouri, 2003). The project will consist of the longitudinal characterization of viral populations evolving in a model of primary cells isolated from blood from donors of different ethnicity. The comparison of properties of these viral populations (fitness, tropism and transmissibility to mosquitoes), in the context of the transcriptomes of cells from different human donors, will reveal the nature and breadth of the constraints due to the different host factors on the viral genomes. The second half of the project will make use of optimized, amplicon free, next generation sequencing (Matranga et al., 2014), to characterize viral populations in samples from patients affected by different degrees of dengue disease severity, as well as in asymptomatic cases. These samples, collected in Cambodia and Senegal in recent years, correspond to an ongoing transcriptomic study in our EU DENFREE consortium. This project will allow the exploration of fundamental questions of evolutionary processes of RNA viruses that propagate as populations of variants, and that are continuously exposed to environments with different constraints. This work aims notably at exploring how host factors can modulate the dynamics of viral population genetic diversity, and the consequences of such variation on key parameters such as pathogenicity (Vignuzzi et al., 2006) or transmissibility to new hosts or species. In addition, the study model for this project is a human pathogen that can cause grave and sometimes lethal symptoms, whose etiology remains poorly understood, and which imposes an increasing burden on public health and economy of many countries.
References:	Guzman, M.G., and Kouri, G. (2003). Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. Journal of clinical virology : the official publication

	of the Pan American Society for Clinical Virology 27, 1-13. Matranga, C.B., Andersen, K.G., Winnicki, S., Busby, M., Gladden, A.D., Tewhey, R., Stremlau, M., Berlin, A., Gire, S.K., England, E., et al. (2014). Enhanced methods for unbiased deep sequencing of Lassa and Ebola RNA viruses from clinical and biological samples. Genome biology 15, 519. Rodenhuis-Zybert, I.A., Wilschut, J., and Smit, J.M. (2010). Dengue virus life cycle: viral and host factors modulating infectivity. Cellular and molecular life sciences : CMLS 67, 2773-2786. Vignuzzi, M., Stone, J.K., Arnold, J.J., Cameron, C.E., and Andino, R. (2006). Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. Nature 439, 344-348.
Expected profile of the candidate:	Experience in molecular biology, virology and interest for bioinformatics

Project number	15
Title of the PhD or postdoctoral project:	Hunting and studying a hybrid metabolic complex in Actinobacteria
Keywords:	Actinobacteria; tuberculosis; Corynebacterium; integrative structural biology; macromolecular complex; metabolism; X-ray crystallography; cryo-EM
Department:	Structural Biology and Chemistry
Name of the lab:	Structural Microbiology
Head of the lab:	Pedro M. Alzari
PhD or Post-doc advisor:	Marco Bellinzoni
Email address:	marco.bellinzoni@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/group-marco-bellinzoni/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	Doctoral school MTCI/Université Paris Diderot (Paris 7)
Research topic	Molecular and cell biology, Microbiology
Presentation of the laboratory and its research topics:	The Structural Microbiology Unit, located at the Institut Pasteur in Paris since 1998 and part of the Structural Biology and Chemistry Department, has a long-lasting interest in the structural biology of mycobacteria. For the last fifteen years, the Unit has been active member of three successive international consortia, funded by the European Commission and dedicated to the identification, characterization and validation of new targets for the development of novel drugs against tuberculosis. The activity of the lab has mostly been focused on the elucidation of the molecular basis of signal transduction in bacteria, with several achievements on the structure and function of Ser/Thr kinases, phosphatases and two-component systems. The lab is now organized in four groups led, respectively, by Pedro M. Alzari (Unit leader), Claudine Mayer, Jean-Christophe Barale and Marco Bellinzoni. The latter group, in which the PhD student will be enrolled, is dedicated to the study of macromolecular complexes in mycobacteria and, more generally, actinobacteria and has contributed to show, in collaboration with several groups abroad, how mycobacteria can tune their central metabolism finely according to the available carbon and nitrogen sources. The group now aims, through the use of cutting-edge technologies, at studying the structure and function of complexes involved in key processes of the bacterial metabolism. A strong multidisciplinary approach that includes microbiology, biochemistry and structural biology techniques is indeed the common theme in the Unit and stays at the heart of our research activities. Elucidating the molecular basis of

	key biological processes to develop new therapeutic strategies is our common goal. The Unit has well-established international collaborations many of which include groups located in South America, e.g. the Pasteur Institute of Montevideo, Uruguay and the Institute of Molecular and Cellular Biology (IBR) in Rosario, Argentina. The lab is also co-organizing, together with R. Brosch's unit, the international 'Tuberculosis 2016' conference at the Institut Pasteur, with more than 500 attendees.
List your five primary research papers:	[1] Wagner, T., Alexandre, M., Duran, R., Barilone, N., Wehenkel, A., Alzari, P.M., and Bellinzoni, M. (2015) The crystal structure of the catalytic domain of the Ser/Thr kinase PknA from M. tuberculosis shows an Src-like autoinhibited conformation. Proteins 83: 982-988. [2] Wagner, T., Barilone, N., Alzari, P.M., and Bellinzoni, M. (2014) A dual conformation of the post-decarboxylation intermediate is associated with distinct enzyme states in mycobacterial KGD (alpha-ketoglutarate decarboxylase). Biochem. J. 457: 425-434. [3] Wagner, T., Bellinzoni, M., Wehenkel, A., O'Hare, H., and Alzari, P.M. (2011). Functional plasticity and allosteric regulation of alpha-ketoglutarate decarboxylase in central mycobacterial metabolism. Chem. Biol. 18: 1011-1020. [4] Bellinzoni, M., Bastard, K., Perret, A., Zaparucha, A., Perchat, N., Vergne, C., Wagner, T., de Melo-Minardi, R.C., Artiguenave, F., Cohen, G.N., Weissenbach, J., Salanoubat, M. and Alzari, P.M. (2011). 3-keto-5-aminohexanoate cleavage enzyme: a common fold for an uncommon Claisen-type condensation. J. Biol. Chem. 286: 27399-27405. Bellinzoni, M., Wehenkel, A., Shepard, W. and Alzari, P.M. (2007) Insights into the mechanism of PPM Ser/Thr phosphatases from atomic resolution structures of a mycobacterial enzyme. Structure 15: 863-872
Description of the project:	The tricarboxylic acid cycle, also known as the Krebs cycle, is one of the very first elucidated biochemical pathways in living organisms. However, despite its supposed universal conservation, new exciting finding like alternative pathways or new ways of regulation have been found and others can still be 'round the corner', especially when we look at the spatial and temporal organization of the enzymes involved. Indeed, a few papers published in the eighties suggested that at least some of the Krebs cycle enzymes might be spatially organized in one or more clusters, likely forming multi-protein complexes [1], although these studies have not found much echo in more recent years. Our interest in this topic started while looking at signal transduction pathways in mycobacteria, when we found how Mycobacterium tuberculosis, the etiological agent of tuberculosis, controls – in a previously unknown way – the activity of the a-ketoglutarate dehydrogenase complex (KDH), involved in the Krebs cycle and devoted to the oxidative decarboxylation of a-ketoglutarate [2,3]. It is worth to note that, in Mtb, components of this complex have been found key for survival in the human host and confer resistance against macrophage generated reactive nitrogen species [4], making them attractive targets for drug development. While we demonstrated that mycobacteria control KDH through the small protein GarA, an unknown way of regulation of central metabolism [2,3,4], we also accumulated evidence that, in mycobacteria and possibly in all Actinobacteria, KDH might actually be merged to PDH, i.e. the pyruvate dehydrogenase complex (which produces acetyl-CoA that enters the Krebs cycle), which is supposed to be composed of three enzymes as well, and structured in a similar way to KDH. In Actinobacteria, therefore, the two complexes are likely to form a sort of unique metabolic 'supercomplex'. Since no KDH-specific E2o component (dihydrolipoamide succinyltransferase) is present, E2p from PDH has to be used as a lipoyl donor instead, and is supposed to make the central core of the supercomplex. Our current challenge is to determine the architecture of

	such a molecular object. The student/fellow will be enrolled in our ongoing work, whose goal is to isolate this complex and/or reconstitute it in vitro, in order to characterize it structurally by an integrative structural biology approach. This approach includes many methodologies that are being carried out through cutting edge equipment to which the group has access, including X-ray crystallography, SAXS (Small Angle X-ray scattering) and cryo-electron microscopy. So far we have been working on Corynebacterium glutamicum as a non-pathogenic model, following two parallel approaches: 'top-down', i.e. isolating the complex from the source, and 'bottom-up', i.e. reconstituting the complex in vitro from the isolated components expressed in recombinant form. Indeed, we have already provided high-resolution X-ray structures of all but one of its component proteins, and multi-protein sub-complexes have been characterized by AUC (analytical ultracentrifugation) and SAXS, in collaboration with the Biophysics platform. Recently, we also succeeded in the reconstitution of the full complex in vitro. The student will therefore have the opportunity to determine this exciting structure and see how the complex works. Our long-term goal, however, is to go beyond a static structural picture, clarifying the dynamic processes by which the different enzymatic activities may be temporally and spatially coordinated, and to understand, in the end, by which molecular mechanisms (and in response to which stimuli) such a huge machinery could be regulated. In turn, his may open exciting perspectives for the development of new antibiotics, keeping in mind that new control mechanisms of the bacterial central metabolism could be unveiled. This project is funded by a 'Young Researcher' grant from the ANR (French National Agency for Research).
References:	[1] Robinson, J.B. and Srere, P.A. (1985) J. Biol. Chem. 260: 10800-10805. [2] Wagner, T., Bellinzoni, M., Wehenkel. A., O'Hare, H.M. and Alzari, P.M. (2011) Chem. Biol. 18: 1011-1020. [3] O'Hare, H.M., Durán, R., Cerveñansky, C., Bellinzoni, M., Wehenkel, A.M., Pritsch, O., Obal, G., Baumgartner, J., Vialaret, J., Johnsson, K., and Alzari, P.M. (2008) Mol. Microbiol. 70: 1408-1423. [4] Ventura, M., Rieck, B., Boldrin, F., Degiacomi, G., Bellinzoni, M., Barilone, N., Alzaidi, F., Alzari, P.M., Manganelli, R. and O'Hare, H.M. (2013) Mol. Microbiol. 90: 356-366. [5] Maksymiuk, C., Balakrishnan, A., Bryk, R., Rhee, K.Y. and Nathan CF. (2015). Proc. Natl. Acad. Sci. USA 112: E5834-5843.
Expected profile of the candidate:	The ideal PhD candidate is a brilliant and enthusiastic master-level student, passionate about structural biology and protein biochemistry and willing to learn working with a portfolio of complementary techniques. Solid communication and presenting skills, as well as capability to work in a team are essential. Documented experience in protein biochemistry, biophysics or structural biology would be an asset.

Project number	16
Title of the PhD:	The tumor suppressor Adenomatous polyposis coli as a regulator of anti-tumor immunity
Keywords:	familial polyposis, colorectal cancer, cytotoxic T cells, anti-tumor immunity
Department :	Immunology
Name of the lab:	Lymphocyte Cell Biology Unit
Head of the lab:	Prof. Andres ALCOVER
PhD or Post-doc advisor:	Dr. Vincenzo DI BARTOLO
Email address:	vincenzo.di-bartolo@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/lymphocyte-cell-biology/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University : ED394 – Physiologie, Physiopathologie et Thérapeutique; Université Pierre et Marie Curie (Paris 6)	
Research topic	Molecular and cell biology, Immunology
Presentation of the laboratory and its research topics:	The Lymphocyte Cell Biology Unit is part of the Immunology Department of the Institut Pasteur. It is also affiliated to the French Institute for Health and Medical Research (Inserm; Unit 1221). The Unit, headed by Pr. Andres Alcover, currently includes 2 staff scientists, a technician, one PhD student and a post-doc. The work of our Unit is at the crossroad of immunology and cell biology. It focuses on T lymphocytes (or T cells) and their role in adaptive immune responses. These cells can detect and fight pathogen infections and cancer. T cells are activated when they recognize 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 not only 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 aim is to understand how immunological synapses are organized at the molecular level and how they control T cell functions. In particular, we investigate the interplay between 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 lymphotropic viruses, such as HIV-1, subvert these cellular mechanisms to favor their replication and spread. We have a longstanding experience in studying cellular and molecular mechanisms underlying T cell function. Some of our work allowed us to characterize the role of cell polarity regulators such as Ezrin and Dlg1 in T cells, showing how these proteins affect cytoskeleton organization at immunological synapses and how they control TCR-induced signaling and gene transcription. More recently, we became interested in the role of another polarity regulator, called Adenomatous Polyposis Coli (APC), a tumor

	suppressor that controls cell activation and differentiation in multiple cell types. Interestingly, APC, Ezrin and Dlg1 work together in some signaling pathways and have been implicated in tumorigenesis, hence their mutations may affect T cells in both physiological and pathological settings.
List your five primary research papers:	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. 15:715-28.
Description of the project:	APC is involved in a familial form of intestinal polyposis and colorectal cancers. Its mutations alter growth and differentiation of intestinal epithelial cells, leading to the development of numerous polyps that later evolve into cancer. APC mutations are also found in the majority of sporadic colorectal cancers. Oncogenic effects of APC are mostly linked to its role in the Wnt/b-catenin signaling pathway. Indeed, APC is a component of the b-catenin degradation complex that controls intracellular levels of this protein and its capacity to regulate transcription of genes involved in cell proliferation and differentiation. APC also regulates cell polarity, cytoskeleton organization and cell migration, that are altered in metastatic cells. Immune cells such as natural killer (NK) and T cells participate in preventing cancer development, hence their abnormalities may support tumorigenesis. Although the function of APC in these cells is unclear, recent work has highlighted alterations of regulatory T cells (Tregs) in mice bearing APC mutations. In line with these data, we found that inhibiting APC expression alters microtubule organization in CD4+ T cells and consequently impairs nuclear translocation and activity of the key transcription factor NFAT (*). Our analysis of ApcMin/+ mutant mice, a model of human polyposis and colorectal cancer, revealed an altered phenotype of Tregs from the intestinal lamina propria. These cells showed reduced nuclear translocation of NFAT, correlating with lower numbers of Tregs producing the anti-inflammatory cytokine IL-10 (*). These data suggest that intrinsic defects of Tregs expressing an APC mutant reduce their ability to control local inflammation linked to altered epithelial cell growth, a condition that would favor tumor development. Based on the data summarized above, we propose that APC mutations may favor

	development of colorectal cancers by affecting not only Tregs but also other immune cell types, including cytotoxic T cells (CTL) or NK cells which are both crucial to control tumor growth. First, defective NFAT-dependent transcription may alter differentiation of CTLs, possibly 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 recognize cancer cells, to secrete cytotoxic granules and to kill them. Finally, since APC control migration of multiple cell types, its mutations may alter CTL or NK migration into tumors. Collectively, these defects would hamper immune surveillance and anti-tumor activity of CTLs and possibly of NK cells, which use similar mechanisms to eliminate tumor cells. Hence, the main goal of the proposed PhD project will be to challenge these hypotheses by addressing the role of APC in normal T cell function and in anti-tumor immune responses. Specific points to be addressed will include the following: 1. Investigate the role of APC in the differentiation of CTLs; 2. Analyze the involvement of APC in CTLs and NK effector functions; 3. Address the role of APC in CTL migration in vitro, in lymphoid organs and in tumor microenvironment. Several experimental models will be available to address these questions, e.g. samples from patients with familial polyposis or colorectal cancers, APC knockdown in human CTLs and T cells from ApcMin/+ mutant mice. Phenotypic studies will include flow cytometry analyses of CTL receptors and differentiation markers. Confocal, TIRF or high-resolution microscopy will assess potential alterations of cytolytic synapses between APC-mutant CTLs and target cells. Functional assays will include measurement of cellular cytotoxicity, transmigration and motility in vitro or in tissue samples. (*) Agüera-Gonzalez, S., Burton, O.T., Vazquez-Chavez, E, Herit, F., Bouchet, J., Lasserre, R., Del Rio-Iñiguez, I., Cuche, C., Di Bartolo, V., Alcover, A. , The polarity regulator Adenomatous polyposis coli ensures NFAT localization to control Treg production of anti-inflammatory cytokines. (Submitted)
References:	McCartney, B.M. and I.S. Nathke, Cell regulation by the Apc protein Apc as master regulator of epithelia. Curr Opin Cell Biol, 2008. 20:186-93. Gounaris, E., N.R. Blatner, K. Dennis, F. Magnusson, M.F. Gurish, T.B. Strom, P. Beckhove, F. Gounari, and K. Khazaie, T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. Cancer Res, 2009. 69:5490-7. Etienne-Manneville, S., APC in cell migration. Adv Exp Med Biol, 2009. 656:30-40. Jenkins, M.R. and G.M. Griffiths, The synapse and cytolytic machinery of cytotoxic T cells. Curr Opin Immunol, 2010. 22:308-13. Martinez, G.J., R.M. Pereira, T. Aijo, E.Y. Kim, F. Marangoni, M.E. Pipkin, S. Togher, V. Heissmeyer, Y.C. Zhang, S. Crotty, E.D. Lamperti, K.M. Ansel, T.R. Mempel, H. Lahdesmaki, P.G. Hogan, and A. Rao, The transcription factor NFAT promotes exhaustion of activated CD8(+) T cells. Immunity, 2015. 42:265-78.
Expected profile of the candidate:	We are looking for a dynamic and creative person with teamwork skills but also able to work autonomously and to bring original contributions to the project. The ideal candidate should have some background in immunology, cell biology or oncology. Practical experience in cell culture, flow cytometry and confocal microscopy or other imaging techniques would be very useful.

Project number	17
Title of the PhD or postdoctoral project:	Proteochemometrics approach to the pharmacological modulation of protein-protein interactions
Keywords:	chemoinformatics, bioinformatics, drug discovery in silico, protein-protein interactions
Department :	Structural biology and chemistry
Name of the lab:	Pole Proteins
Head of the lab:	Bernard Delmas
PhD or Post-doc advisor:	Olivier Sperandio
Email address:	olivier.sperandio@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/proteins/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	ED MTCI n°563, University Paris Diderot
Research topic	Bio-informatics
Presentation of the laboratory and its research topics:	The missions of the Pole Proteins are to provide to the research community, in priority that of Institut Pasteur and its international network, efficient and cutting-edge tools, technologies and expertise to enable the production and the characterization of proteins and their macro-molecular assemblies, in order to support fundamental research in biology as well as to assist the development of novel diagnostic and treatment strategies, in particular against infectious diseases, from vaccination to therapeutic approaches. The Pole Proteins is built around four core facilities, the Recombinant Protein Production (PFPR), Antibody Engineering (PFIA), Molecular Biophysics (PFBMI) and Crystallography (PFC) facilities and a research group “Chemoinformatics and Protechemometrics” (C&P) headed by Olivier Sperandio dedicated to the study of protein-protein interactions using chemical biology, chemoinformatics and proteochemometrics. Protein-protein interactions play an essential role in nearly all biological processes and their deregulation is often associated with disease states. For this reason, there is a growing interest to target them for therapeutic interventions using low-molecular-weight compounds (<1000 g/mol). The chemoinformatics and protechemometrics team (C&P) within the Pole Proteins and headed by Dr Olivier Sperandio is dedicated to the use of in silico approaches that facilitate the pharmacological modulation by small molecules of macromolecular interactions such as protein-protein interactions (PPI). The C&P team combines chemoinformatics, proteochemometrics and structural bioinformatics techniques to characterize the structural properties of binding cavities present at the core of PPI interfaces and the most suitable physicochemical profiles of the small molecules meant to modulate them.

List your five primary research papers:	1. Imbalance in chemical space: How to facilitate the identification of protein-protein interaction inhibitors. Kuenemann MA, Labbé CM, Cerdan AH, Sperandio O. Sci Rep. 2016 Apr 1;6:23815. 2. iPPI-DB: an online database of modulators of protein-protein interactions. Labbé CM, Kuenemann MA, Zarzycka B, Vriend G, Nicolaes GA, Lagorce D, Miteva MA, Villoutreix BO, Sperandio O. Nucleic Acids Res. 2016 Jan 4;44(D1):D542-7. 3. Stabilization of protein-protein interaction complexes through small molecules. Zarzycka B, Kuenemann MA, Miteva MA, Nicolaes GA, Vriend G, Sperandio O. Drug Discov Today. 2016 Jan;21(1):48-57. 4. Which three-dimensional characteristics make efficient inhibitors of protein-protein interactions? Kuenemann MA, Bourbon LM, Labbé CM, Villoutreix BO, Sperandio O. J Chem Inf Model. 2014 Nov 24;54(11):3067-79. 5. Identification of novel small molecule inhibitors of activated protein C. Sperandio O, Wildhagen KC, Schrijver R, Wielders S, Villoutreix BO, Nicolaes GA. Thromb Res. 2014 Jan 25. pii: S0049-3848(14)00050-4.
Description of the project:	An important aspect of our work relies on the rationalization of the chemical space of protein-protein interactions' inhibitors by analyzing the properties of successful examples of pharmacological PPI modulations. To this end, we are driving the iPPI-DB initiative project (http://www.ippidb.cdithem.fr/), a database of PPI modulators (only small molecules). This database is a great source of pharmacological data that help us to derive some trends about the PPI chemical space using chemoinformatics and machine learning techniques. Another key aspect of our project is also to get some insight into the PPI interfaces' properties themselves that obviously condition the chemotypes of small compounds which may or may not bind to them. By crossing both types of information, i.e by crossing the target and the chemical spaces of PPI, we aim at determining which privileged chemotypes and chemical structures should be associated with which types of PPI targets. The final goal is therefore to facilitate the identification of quality chemical probes on PPI targets and more generally on macromolecular interactions by the mean of complementing chemical biology and drug discovery approaches. The objective of the proposed PhD project is to push forward these approaches in the context of a large scale analysis on all available structural data regarding both chemical structures of PPI modulators (small compounds) and of PPI interfaces (3D structures of the targets). The first part of the project will consist of updating the data of our iPPI-DB database by collecting pharmacological data from the literature. New functionalities will be made accessible to the user and new PPI targets will be represented in order to cover an even more representative region of chemical space. The purpose at this stage will be to provide ourselves with an up-to-date quality dataset of PPI modulators. Such an update will be published in the database issue of Nucleic Acid Research as previously made recently [Labbé, 2016]. The dataset will allow the PhD candidate to carry out an in-depth big data analysis of the PPI chemical space with chemoinformatics methods, in terms of physicochemical profiles, clustering, and privileged substructures. Conversely, the Protein Data Bank (PDB) and other specialized online databases will be used to collect structural data of known PPI targets both having cocrystallized modulators and also apo-structures without any bound ligands. Structural bioinformatics tools will be used to scrutinize those protein interfaces in order to identify the properties of the PPI targets required to bind small molecules. By crossing both types of analyses (modulators versus PPI interfaces) we will be in the position of making educated predictions at proteomics scale. The purpose of the project is therefore to set on a robust procedure, easy-to-use, to evaluate the potential of a PPI target and the type of privileged chemical structures it requires for ligand-binding by exclusively analyzing the structural properties of their interfaces. This will serve to prioritize the most promising PPI targets to be investigated pharmacologically for therapeutics interventions and with which type of chemistry, with numerous

	applications in drug discovery and chemical biology. At least two PPI targets will be selected as a proof of concept for the developed procedure. The immersion of Sperandio's group in the Pole Proteins will dramatically help on this matter as the PhD candidate will have access to the full ressources of the platforms in the Pole Proteins in terms protein purification, biophysical methods and Xray crystallography.
References:	Labbé CM, Kuenemann MA, Zarzycka B, Vriend G, Nicolaes GA, Lagorce D, Miteva MA, Villoutreix BO, Sperandio O. iPPI-DB: an online database of modulators of protein-protein interactions. Nucleic Acids Res. 2016 Jan 4;44(D1):D542-7.
Expected profile of the candidate:	Ideal PhD candidates should have a background in chemoinformatics or structural bioinformatics. Experience in biostatistics would be an asset in the project. Knowledge with the Linux/Unix environments will greatly help as well.

Project number	21
Title of the PhD or postdoctoral project:	Understanding cell fate choice during human sex determination
Keywords:	Sex Determination, cell-fate choice, whole genome sequencing, functional assays, cellular reprogramming
Department:	Developmental and Stem Cell Biology
Name of the lab:	Unit of Human Developmental Genetics
Head of the lab:	Ken McElreavey
PhD or Post-doc advisor:	Ken McElreavey
Email address:	kenmce@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/human-developmental-genetics/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University	UPMC, ED394; Physiologie, Physiopathologie et Therapeutique
Research topic	Molecular and cell biology, Medicine, Genetics, Health, environment, society
Presentation of the laboratory and its research topics:	Human gonad development depends on a cell fate decision that occurs in the bipotential anlage to commit to either Sertoli (male) or granulosa (female) cells. Current data suggest that mammalian sex-determination (SD) involves complex mutually antagonistic genetic interactions of testis- and ovary-determining pathways. Uniquely in development, SD is achieved by suppression of the alternate fate and this suppression is maintained in adulthood. Although many early genetic, cellular and morphological events during gonadal development have been characterized, the molecular mechanisms involved in human SD are poorly understood. There are several reasons for this. First, there are no powerful and informative cellular models of sex-determination. No cell line has been established with all the properties of Sertoli cells, the first cell lineage to form in the embryonic testis. Primary immature and mature Sertoli cells as well as established cell lines lose their characteristics during prolonged culture. Second, familial cases with errors in sex-determination (XY females or XX males) are very rare. This impedes classical genetic studies to identify genes involved in the process. Third, sex-determination is not conserved in evolution. Model organisms, such as

	Drosophila and C. elegans use different molecular strategies to determine sex. In zebrafish, the mechanism is thought to involve multiple, as yet unidentified, genes. In other fish species, sex is determined by interferon regulatory factor 9 through unknown mechanisms, whereas in birds sex is determined by the DMRT1 gene. In mammals the initial events of sex determination are genetically determined (XX female or XY male). SRY on the human Y chromosome is the master regulator switch that triggers the formation of the testes in mammals. However, the plasticity of the system is highlighted by some rodent species that do not have a Y chromosome nor an SRY gene. The aim of the unit of Human Developmental Genetics is to understand the genes and mechanisms involved in directing cell fate choice in the developing human gonad. To achieve this we have developed a large collection of biological material from patients with sex-reversal or infertility, through collaborations with clinical centres worldwide. Our research activities exploit this unique biological resource that has been used to make a number of major discoveries in the field, to identify novel factors/genes involved in human SD. To identify the mechanism by which these genes impact cell fate choice, we are developing novel cellular models including, but not limited to, those based on biomaterial from patients with disorders of sex development (DSD) and their unaffected family members.
List your five primary research papers:	Bashamboo A, Donohoue PA, Vilain E, Rojo S, Calvel P, Seneviratne SN, Buonocore F, Barseghyan H, Bingham N, Rosenfeld JA, Mulukutla SN, Jain M, Burrage L, Dhar S, Balasubramanyam A, Lee B; Members of UDN, Eozenou C, Suntharalingham JP, de Silva K, Lin L, Bignon-Topalovic J, Poulat F, Lagos CF, McElreavey K, Achermann JC. A recurrent p.Arg92Trp variant in steroidogenic factor-1 (NR5A1) can act as a molecular switch in human sex development. Hum Mol Genet. 2016 Jul 4. pii: ddw186. Murphy MW, Lee JK, Rojo S, Gearhart MD, Kurahashi K, Banerjee S, Loeuille GA, Bashamboo A, McElreavey K, Zarkower D, Aihara H, Bardwell VJ. wer D, Aihara H, Bardwell VJ. An ancient protein-DNA interaction underlying metazoan sex determination. Nat Struct Mol Biol. 2015 Jun;22(6):442-51 Lourenço D, Brauner R, Rybczynska M, Nihoul-Fékété C, McElreavey K, Bashamboo A. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1597-602. Bashamboo A, Ferraz-de-Souza B, Lourenço D, Lin L, Sebire NJ, Montjean D, Bignon-Topalovic J, Mandelbaum J, Siffroi JP, Christin-Maitre S, Radhakrishna U, Rouba H, Ravel C, Seeler J, Achermann JC, McElreavey K. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. Am J Hum Genet. 2010 Oct 8;87(4):505-12. Lourenço D, Brauner R, Lin L, De Perdigo A, Weryha G, Muresan M, Boudjenah R, Guerra-Junior G, Maciel-Guerra AT, Achermann JC, McElreavey K, Bashamboo A. Mutations in NR5A1 associated with ovarian insufficiency. N Engl J Med. 2009 Mar 19;360(12):1200-10.
Description of the project:	Human SD is an unusual biological process that is regulated by a double repressive system where an equilibrium of mutually antagonistic pathways (primarily SOX9 vs WNT/B-CAT) must be attained for normal development of either the testis or ovaries. Changes in this delicate balance results in DSD or infertility. We have performed exome sequencing on 60 cases of XY gonadal dysgenesis and 50 cases of XX

	TDSD/OTDSD. This has already led to the identification of new genetic causes of DSD (see publications). We have several novel candidates (negative regulators of WNT) with mutations that require detailed functional analysis to understand their role in testis-determination in XX chromosomal context. The objectives of the current proposal is to 1- Characterise mutants in the negative regulators of WNT signalling and understand the role of these genes in testis-determination in a female. 2- Identify novel genetic factors involved in human sex-determination by performing whole genome sequencing on a series of rare cases of sex-reversal. 3- Develop novel cellular models to understand how the human male Sertoli cells can form in a female XX background. 1.Characterise mutants in the negative regulators of WNT signalling 1.1. Gene expression in the human gonad at sex-determination: There is a considerable public data available on gene expression during mouse SD, however there is very little information available on the human. This is important since we have previous shown that both SRY and NR5A1 have different embryonic expression profiles from the orthologous genes in the mouse. This project aims to characterize the expression of candidate genes identified by NGS (see above) in human fetal ovary and testis tissue (6-9 weeks post conception) provided with approval from the CHU CEOS, Rennes. 1.2. Effect of mutations on WNT signaling pathway: The mutations identified in proteins known to repress the canonical WNT signaling pathway will be assessed for their effect on WNT signaling using the TOPFlash-TCF assay. The protein-protein interaction between the mutant and WT proteins and the WNT protein partners will be analysed using methodologies routinely performed in the lab (see publications). 1.3. Transcriptional changes in SD gene expression in response to mutant proteins: To study of alterations in the global gene expression in somatic cells of the ovaries as an affect of these mutations the project will use an ex-vivo transcriptional profiling approach. This would be performed using a model developed in the lab, which involves micromass culture of female gonad total cells from SF1/eGFP transgenic mouse (see publications). 2. Whole Genome sequencing The unit has been performing exome sequencing on patients with DSD. This has been very informative in revealing novel causes of human sex determination (see publications). However, 50% of all cases remain unexplained, particularly the 46,XX TDSD/OTDSD phenotype (unpublished data). We will perform whole genome sequencing (30X coverage) in these cases. The novel variants will be identified and further characterised using a battery of in-silico, in-vitro and in-vivo assays (see publications) dependent on the nature of the protein and mutations. 3. Novel cellular models To understand the mechanism of formation of testis in XX chromosomal context, the project aims to use ovarian granulosa carcinoma (KGN). These cells recapitulate the transcriptome and molecular milieu of the developing granulosa cells. Using this

	model we have already shown that a missense heterozygous mutation identified in an XX male, has the capacity to induce endogenous « male/Sertoli» programme when introduced in the female/granulosa cells. This model will be used to assay the effects of the mutations identified in the lab in association with 46, XX TDSD/OTDSD. This would include the mutations already identified by us in the negative regulators of WNT signalling, and any future mutations to be identified using Genome sequencing.
References:	Bashamboo A, McElreavey K. Human sex-determination and disorders of sex-development (DSD). Semin Cell Dev Biol. 2015 Sep;45:77-83. doi: 10.1016/j.semcdb.2015.10.030. Epub 2015 Oct 23. Hyon C, Chantot-Bastaraud S, Harbuz R, Bhouri R, Perrot N, Peycelon M, Sibony M, Rojo S, Piguel X, Bilan F, Gilbert-Dussardier B, Kitzis A, McElreavey K, Siffroi JP, Bashamboo A.Refining the regulatory region upstream of SOX9 associated with 46,XX testicular disorders of Sex Development (DSD). Am J Med Genet A. 2015 Aug;167A(8):1851-8. doi: 10.1002/ajmg.a.37101. Epub 2015 Apr 21. Tobias ES, McElreavey K. Next generation sequencing for disorders of sex development. Endocr Dev. 2014;27:53-62. doi: 10.1159/000363615. Epub 2014 Sep 9. Review. Lucas-Herald AK, Bashamboo A. Gonadal development. Endocr Dev. 2014;27:1-16. doi: 10.1159/000363608. Epub 2014 Sep 11. Review. Bashamboo A, Brauner R, Bignon-Topalovic J, Lortat-Jacob S, Karageorgou V, Lourenco D, Guffanti A, McElreavey K. Mutations in the FOG2/ZFPM2 gene are associated with anomalies of human testis determination. Hum Mol Genet. 2014 Jul 15;23(14):3657-65. doi: 10.1093/hmg/ddu074. Epub 2014 Feb 18.
Expected profile of the candidate:	The candidate must have experience in Bioinformatics analysis, particularly of large datasets from the eukaryotes. An experience of standard molecular biology techniques and assays, culture of primary and established cell lines and cellular reprogramming is highly desirable but not essential. The candidate must have fluency in spoken and written english and an aptitude for quick learning. They must have the ability to work as a part of the team.

Project number	22
Title of the PhD or postdoctoral project:	Exploring the links between hepatitis C virus (HCV) genetic variability and virus-induced metabolic disorders
Keywords:	hepatitis C virus, genotype, steatosis, diabetes, lipid metabolism, interactomics
Department:	Virology
Name of the lab:	Molecular Genetics of RNA Viruses
Head of the lab:	Pr. Sylvie van der Werf
PhD or Post-doc advisor:	Dr. Annette Martin
Email address:	annette.martin@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/fr/team/molecular-genetics-of-rna-viruses/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe), Post-Doc (2 years at your lab). Available ONLY for Mexican researchers who already have a working contract in their home country
Doctoral school affiliation and University	Bio Sorbonne Paris Cité (BioSPC), Paris Diderot University
Research topic	Virology
Presentation of the laboratory and its research topics:	The laboratory of Molecular Genetics of RNA Viruses associates three research groups on influenza viruses and one research group on hepaciviruses, dealing with two categories of major human viral pathogens responsible for worldwide pandemics. The research interests of the Hepacivirus / Host Interactions group that will host the PhD student focus on human hepatitis C virus (HCV), as well as a growing number of phylogenetically related viruses recently identified in various mammal species. Among these viruses, HCV and GB virus B (GBV-B) are both responsible for acute self-resolving or chronic hepatitis. While HCV is known to only infect humans and chimpanzees, GBV-B infects small New World primates (tamarins, marmosets). Our past and current research has aimed to comparatively characterize the life cycles of HCV, GBV-B and other hepacivirus (entry, genome replication, particle assembly) and determine whether they share common properties or exhibit differences that may translate into the identification of determinants responsible for the host species tropism of these hepaciviruses (1-3, 5). This is ultimately meant to help develop an immunocompetent small primate model of HCV infection, that would be valuable for the development of an hepatitis C vaccine. In this context, nonstructural protein 2 (NS2), a key viral protein which is involved in both the polyprotein cleavage that releases the replication complex and particle assembly has been the focus of our most recent research projects (4). In an effort to understand the mechanisms of HCV carcinogenesis, another current research axis of the group addresses HCV interference and notably genotype-specific

	interference with host signaling pathways resulting in the transcriptional modulation of genes involved in cell cycle regulation (Aicher et al., in preparation).
List your five primary research papers:	1. Warter L, Cohen L, Benureau Y, Chavez D, Yang Y, Bodola F, Lemon SM, Traboni C, Lanford RE, Martin A. 2009. A cooperative interaction between nontranslated RNA sequences and NS5A protein promotes in vivo fitness of a chimeric hepatitis C/GB virus B. PLoS ONE 4:e4419. 2. Benureau Y, Warter L, Malcolm BA, Martin A. 2010. A comparative analysis of the substrate permissiveness of HCV and GBV-B NS3/4A proteases reveals genetic evidence for an interaction with NS4B protein during genome replication. Virology 406:228-240. 3. Krey T, d'Alayer J, Kikuti CM, Saulnier A, Damier-Piolle L, Petitpas I, Johansson DX, Tawar RG, Baron B, Robert B, England P, Persson MA, Martin A, Rey FA. 2010. The disulfide bonds in glycoprotein E2 of hepatitis C virus reveal the tertiary organization of the molecule. PLoS Pathog. 6:e1000762. 4. Boukadida C, Marnata C, Montserret R, Cohen L, Blumen B, Gouttenoire J, Moradpour D, Penin F, Martin A. 2014. NS2 Proteins of GB Virus B and Hepatitis C Virus Share Common Protease Activities and Membrane Topologies. J. Virol. 88:7426-7444. 5. Marnata C, Saulnier A, Mompelat D, Krey T, Cohen L, Boukadida C, Warter L, Fresquet J, Vasiliauskaite I, Escriou N, Cosset FL, Rey FA, Lanford RE, Karayiannis P, Rose NJ, Lavillette D, Martin A. 2015. Determinants Involved in Hepatitis C Virus and GB Virus B Primate Host Restriction. J. Virol. 89:12131-12144.
Description of the project:	Hepatitis C virus (HCV) infection is a main cause of chronic liver inflammation in approximately 3% of the population worldwide, leading to fibrosis, cirrhosis and hepatocellular carcinoma (HCC), as well as major metabolic disorders such as steatosis, an accumulation of fat in the liver, and insulin resistance (1). HCV is characterized by extensive genetic diversity with 7 genotypes and many subtypes thought to be associated with varying clinical outcomes. In particular, HCV genotype 3 chronic infections have been reported to be associated with higher steatosis prevalence and increased fibrosis progression rate toward cirrhosis and HCC (2). Because of these pathological characteristics, but also due to its relative resistance to the direct acting antivirals (DAAs) recently licensed and to its high prevalence in drug users in Europe, HCV genotype 3 stands out as a major health issue that needs to be addressed (3). HCV Core protein, a component of HCV particle, does not only have a critical role in virion assembly, but has also been reported in overexpression systems to be responsible for the modulation of host transcription machinery, signaling pathways, lipid metabolism, apoptosis, as well as cell cycle perturbation and mitochondrial dysfunction (4, 5). The proposed project aims at clarifying the role of HCV Core protein and the impact of Core genotypic/ subgenotypic variability in the regulation of cellular lipid and glucose metabolism pathways. Importantly, these studies will be carried out in relevant HCV infection systems. The PhD student will generate new recombinant viruses derived from a highly replicating HCV strain of subtype 2a and expressing Core sequences from clinical isolates of various genotypes, notably genotype 3, associated with different pathogenic signatures (steatosis grade, insulin-resistance, etc.). Using these recombinant viruses, he/she will study the interplay between HCV Core variants and the regulation of lipid metabolic pathways in infected hepatic cells. These studies will involve the monitoring of the regulation of cellular genes that are essential for cholesterol and fatty acid biosynthesis, fatty acid ß-oxydation, glucose uptake,

	lipoprotein production and secretion. The extent of the association of Core with lipid droplets in infected cells will be analyzed through quantitative co-localization immunofluorescence analyses and electron microscopy approaches. Selected core recombinant viruses will be further refined to express a tagged version of core, which will serve to identify host factors that differentially interact with core variants in hepatoma cells. Toward this goal, a high throughput mass spectrometry screen relying on combined liquid chromatography and tandem mass spectrometry analysis (LC MS/MS) will be performed according to a methodology already set in the laboratory. This will provide a list of core interactors in infected hepatoma cells. The importance of the identified host factors in HCV metabolic dysregulation will be further studied by functional approaches relying on HCV variants with defined phenotypes. HCV interplay with selected host factors identified in this study may then be confirmed in infected primary human hepatocyte cultures, as well as using samples from patient cohorts. Overall, these studies are expected to shed light on the molecular mechanisms underlying the association of HCV genomic variability with virus-induced metabolic disorders and help identify disease progression markers.
References:	1. Yamane D, McGivern DR, Masaki T, Lemon SM. 2013. Liver injury and disease pathogenesis in chronic hepatitis C. Curr. Top. Microbiol. Immunol. 369:263-288. 2. Probst A, Dang T, Bochud M, Egger M, Negro F, Bochud PY. 2011. Role of hepatitis C virus genotype 3 in liver fibrosis progression--a systematic review and meta-analysis. J. Viral Hepat. 18:745-759. 3. Goossens N, Negro F. 2014. Is genotype 3 of the hepatitis C virus the new villain? Hepatology 59:2403-2412. 4. Jones DM, McLauchlan J. 2010. Hepatitis C virus: assembly and release of virus particles. J. Biol. Chem. 285:22733-22739. 5. Lin MV, King LY, Chung RT. 2015. Hepatitis C virus-associated cancer. Annual review of pathology 10:345-370.
Expected profile of the candidate:	Prior experience in cell culture and handling of infectious agents will be an asset.

Project number	23
Title of the PhD or postdoctoral project:	Plasmodium vivax, relapses, genotyping, serology
Keywords:	Plasmodium vivax, relapses, genotyping, molecular epidemiology, short amplicon sequencing
Department:	Parasites and Insect Vectors
Name of the lab:	Malaria: Parasites & Hosts
Head of the lab:	Ivo Mueller
PhD or Post-doc advisor:	Ivo Mueller
Email address:	ivo.mueller@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/en/team/malaria-parasites-and-hosts/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	I plan to have my association with UPMC
Research topic	Parasitology, Bio-informatics, Epidemiology, Genetics, Infectious diseases
Presentation of the laboratory and its research topics:	The Malaria: Parasites & Hosts Unit specializes in studying the complex interaction between Plasmodium parasites and its human and vector hosts using a combination of well-defined population-based studies in endemic countries with in-depth molecular, serological and systems biology studies. With these approaches we aim to gain a better understanding of i) Parasite dynamics within the human host and the genetic diversity and evolutionary history of parasites, ii) differential patterns of natural acquisition of immune responses and their association with parasite exposure and protection from infection and iii) the transmission epidemiology and biology of Plasmodium gametocytes and sporozoites. A key interest our unit lies in the study of P. vivax with a particular focus on understanding the biology, epidemiology and control / prevention of relapsing P. vivax infections. The research in our unit is multi-disciplinary and highly collaborative and involves close interactions with researchers and communities in malaria endemic countries.
List your five primary research papers:	Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the Plasmodium vivax and Plasmodium ovale hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS medicine. 2015;12(10):e1001891. Waltmann A, Darcy AW, Harris I, Koepfli C, Lodo J, Vahi V, et al. High Rates of Asymptomatic, Sub-microscopic Plasmodium vivax Infection and Disappearing

	Plasmodium falciparum Malaria in an Area of Low Transmission in Solomon Islands. PLoS neglected tropical diseases. 2015;9(5):e0003758. Mueller I, Schoepflin S, Smith TA, Benton KL, Bretscher MT, Lin E, et al. Force of infection is key to understanding the epidemiology of Plasmodium falciparum malaria in Papua New Guinean children. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(25):10030-5. Senn N, Rarau P, Stanisic DI, Robinson L, Barnadas C, Manong D, et al. Intermittent preventive treatment for malaria in Papua New Guinean infants exposed to Plasmodium falciparum and P. vivax: a randomized controlled trial. PLoS Medicine. 2012;9(3):e1001195. Rosanas-Urgell A, Lin E, Manning L, Rarau P, Laman M, Senn N, et al. Reduced risk of Plasmodium vivax malaria in Papua New Guinean children with Southeast Asian ovalocytosis in two cohorts and a case-control study. PLoS Medicine. 2012;9(9):e1001305.
Description of the project:	After a decade of significant gains in the control of malaria, P. vivax in now the dominant parasite throughout the Americas. This is largely due the ability of P. vivax parasites to relapse from long-lasting liverstages. Key to its elimination is therefore the ability to not detect and treat people with asymptomatic blood-stage and those that carry dormant liver-stage infections. The aim of this PhD project is to i) develop novel genotyping methods that allow differentiating between relapses and new infections, determine transmission networks and ii) use novel serological markers of recent exposure to P. vivax infection to confirm ongoing local transmission and identify people at high risk of relapsing P. vivax infections in longitudinal studies conducted in Peru by Dr. Dionicia Gamboa from the Universidad Peruana Gayetano Heredia in Lima, Peru. We recently demonstrated that relapses account for 80% of all vivax bloodstage infections (Robinson, PLoS Medicine 2015, 12(10):e1001891). Due to the lack of a diagnostic test for relapses, little is however know what determine patterns of relapse, if they are triggered or how they contribute to transmission. A primary infections and its relapses are often meiotic siblings or half-siblings (Bright, PLoS NTD 2014, 8(6):e2882). We will exploit this to develop an amplicon-based, multi-locus genotyping assay that will allow us to differentiate relapses from primary infections. Whole genome sequence will be investigated to identify both highly polymorphic short amplicons and a specific set of SNP. Next generation sequencing assay will the be developed for high-throughput genotyping of field samples and specific bioinformatics programs applied to detected the genetic relatedness between concurrent and subsequent infections within and between individuals in a community. These genetic studies will be supplemented by measuring antibodies to novel panel of P. vivax antigens and the presence of these antibodies will be correlated with the risk of experiencing a P. vivax relapse in the next 6 months. This project will require a combination of laboratory (molecular biology & serology), bioinformatics and epidemiology techniques and will conducted in close collaboration with and co-supervision by Dr. Dionicia Gamboa from the Universidad Peruana Gayetano Heredia in Lima, Peru.
References:	Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, et al. Strategies for understanding and reducing the Plasmodium vivax and Plasmodium ovale

	hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Medicine. 2015;12(10):e1001891 Bright AT, Manary MJ, Tewhey R, Arango EM, Wang T, Schork NJ, et al. A high resolution case study of a patient with recurrent Plasmodium vivax infections shows that relapses were caused by meiotic siblings. PLoS Negl Trop Dis. 2014;8(6):e2882. Koepfli C, Colborn KL, Kiniboro B, Lin E, Speed TP, Siba PM, et al. A high force of plasmodium vivax blood-stage infection drives the rapid acquisition of immunity in papua new guinean children. PLoS Negl Trop Dis. 2013;7(9):e2403. White MT, Karl S, Battle KE, Hay SI, Mueller I, Ghani AC. Modelling the contribution of the hypnozoite reservoir to Plasmodium vivax transmission. eLife. 2014;3.
Expected profile of the candidate:	Experience in molecular biology and infectious diseases Interest in Epidemiology, biostatistics and bioinformatics

Project number	24
Title of the PhD or postdoctoral project:	Machine Learning in computational pathology: application in breast cancer diagnosis
Keywords:	Computation pathology, histology, breast cancer diagnosis, machine learning
Department:	Cell Biology and Infection
Name of the lab:	Bioimage Analysis Unit
Head of the lab:	Jean-Christophe Olivo-Marin
PhD or Post-doc advisor:	Jean-Christophe Olivo-Marin
Email address:	jcolivo@pasteur.fr
Web site address of the lab:	https://research.pasteur.fr/fr/team/bioimage-analysis/
Your proposal refers to	Full PhD at your lab (3 years at Institut Pasteur, IP Guyane or IP Guadeloupe)
Doctoral school affiliation and University	EDITE Université Paris Descartes
Research topic	Molecular and cell biology, Bio-informatics, Health, environment, society
Presentation of the laboratory and its research topics:	The scientific project of the Bioimage Analysis (BIA) unit is to develop image analysis and computer vision tools for the processing and quantification of biological images. Our work over the last years has been centered on developing new algorithms for multi-particle tracking, active contours models, PSF approximations for image deconvolution and colour image analysis. It has resulted in powerful tools for spot detection and counting in real-time imaging of virus and genes, movement and shape analysis in 3D+t microscopy and histological biopsies analysis. These methods and algorithms are made available to biological groups with which we collaborate in a large number of projects.
List your five primary research papers:	- Lagache, T., Sauvonnet, N., Danglot, L., and Olivo-Marin, J.-C . (2105) Statistical analysis of molecule colocalization in bio-imaging, Cytometry A , 87, 6, pp.568-79Microscopy, IEEE Journal of Selected Topics in Signal Processing , 10, 1, pp. 3-5 - Chenouard, N., Bloch, I., and Olivo-Marin, J.-C. (2013) Multiple Hypothesis Tracking for Cluttered Biological Image Sequences, IEEE Trans. Pattern Analysis and Machine Intelligence , 35, 11, pp. 2736-50 - de Chaumont F, Dallongeville S, Chenouard N, Hervé N, Pop S, Provoost T, Meas-Yedid V, Pankajakshan P, Lecomte T, Le Montagner Y, Lagache T, Dufour A, and Olivo-Marin, J.-C. (2012) Icy: an open bioimage informatics platform for extended reproducible research, Nature Methods , 9, 7, 690-6 -de Chaumont, F., Dos-Santos Coura, R., Serreau, P., Cressant, A., Chabout, J., Granon, S. and Olivo-Marin, J.-C. (2012) Computerized video analysis of social interactions

	between mice, Nature Methods, 9, 4, 410-7 - Meas-Yedid V, Servais A, Noël LH, Panterne C, Landais P, Hervé N, Brousse N, Kreis H, Legendre C, Thervet E, Olivo-Marin JC, Morelon E. Transplantation. 2011;92:890-9 - Zimmer, C., Labruyère, E., Meas-Yedid, V., Guillén, N. and Olivo-Marin, J.-C. (2002) Segmentation and tracking of migrating cells in videomicroscopy with parametric active contours : a tool for cell-based drug testing, IEEE Trans. on Medical Imaging , 21, 10, 1212-21.
Description of the project:	The breast cancer (BC) disease is considered to be the second cause of death worldwide and its incidence had been growing as a result of increasing exposure to risk factors and life expectancy. The development of breast cancer involves a progression through series of intermediate processes, starting with ductal hyperproliferation, followed by subsequent evolution to carcinoma in situ, invasive carcinoma, and finally into metastatic disease. This high heterogeneity has been rated and used as a tool to help in treatment and prognosis of the patients. The histological classification is depending on the origin location. The ductal tumours develop in breast ducts and represent 80% of the samples. The lobular tumours develop inside the lobes and account for 10 to 15% of cases. The staging system to classify BC tumours is the Tumour-Node-Metastasis classification of malignant tumours, which is recommended by the Union for International Cancer Control. Given the high variability in clinical progression of disease, the identification of markers that could predict tumour behaviour is particularly important. Actually, the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor type 2 (HER2) has been used as predictive markers for identifying a high-risk phenotype and for selection of the most efficient therapies. The utility of ER, PR and HER2 is well accepted for infiltrating ductal carcinoma (IDC) and it is recommended that their status be determined on all invasive carcinomas. The use of these markers by the IDC exemplifies the potential of molecular biomarkers in guiding clinical decisions. Already, the status of these markers helps determine which patients are likely to respond to targeted therapies. The most commonly used technique to evaluate tissue-based biomarkers is immunohistochemistry, owing to its relatively low cost and increased time efficiency over other methods, such as ligand-binding assays, or fluorescence in situ hybridization. Immunohistochemistry assessment is typically performed by pathologists using optical microscopy. While the ability of pathologists to interpret histomorphological characteristics, such as whether a tissue is cancerous, is extremely reliable, human interpretation of quantitative image features appears more difficult. Measuring the number of cells positive for a specific biomarker or quantifying the proportion of area, and quantifying the intensity of biomarker stains, may suffer from significant intra and inter-observer variability. However, objective and accurate assessment, especially in case of the predictive biomarkers, is highly relevant because therapeutic decisions rely on the quantitative scoring result. Therefore, pathologists and clinicians need for accurate biomarker quantification tools that can support treatment decisions. In such assessment the reproducibility is still a key issue. In fact, colour and intensity can vary a lot in histopathology images due to several factors: the sample, the protocol of slide preparation (staining), and image acquisition setup. In the present work, we propose to develop a computer-assisted diagnostics system based on the machine learning approach that helps to classify histological samples from breast cancer. We will focus on the convolutional neural network also known as deep learning method, which is efficient to capture high variation in the data. With this, we should be able to determine in a quantitative, efficient and reproducible way

	the amount of the ER and PR in the slides. In fact, the digital image analysis should increase the capacity, precision and accuracy compare to visual evaluation or counting, used in pathology diagnosis and research. Hence these new methods potentially will save pathologist time and resources, and produce a more objective assessment in order to help the understanding of breast cancer by providing insights into functional and molecular genetic characterization of tumours.
References:	- Polyak K. J Clin Invest. 2007 Nov;117(11):3155-63 - Singletary SE, Greene FL; Breast Task Force. Semin Surg Oncol. 2003;21:53-9 - Weigelt B, Reis-Filho JS. Breast Cancer Res. 2010 Dec 20;12 Suppl 4:S5 - Maughan KL, Lutterbie MA, Ham PS. Am Fam Physician. 2010;81:1339-46 - Keller B, Chen W, Gavrielides MA. Arch Pathol Lab Med. 2012;136:539-50.
Expected profile of the candidate:	The required candidate, should have skills in computer science, applied mathematics or related areas and should be interested in biology and medecine. Due to the fact that the fundamental base of this proposal is multidisciplinary between computer science and biology, the candidate will be trained with the entire set of approaches in both fields.