Full Ph.D. PROPOSAL FOR THE EMHE PROGRAM

(October 2018-September 2021)

Project #	Department	Lab name/Head	Project name	Advisor	Lab website
1	Cell Biology and Infection	**Cell polarity, migration and cancer lab** (Sandrine ETIENNE-MANNEVILLE)	Role of intermediate filaments in mechanotransduction during cell migration	Sandrine ETIENNE-MANNEVILLE setienne@pasteur.fr	https://research.pasteur.fr/en/team/cell-polarity-migration-and-cancer/
2	Cell Biology & Infection	**Cellular Biology of Microbial Infection** (Agathe SUBTIL)	Functional study of the TIFA-dependent innate immune response during Chlamydia trachomatis infection	Yongzheng WU yongzheng.wu@pasteur.fr	https://research.pasteur.fr/en/team/cellular-biology-of-microbial-infection/
3	Cell Biology & Infection	**Imaging and Modeling Unit** (Christophe ZIMMER)	Super-resolution imaging and computational modeling of dynamic chromosome architecture	Christophe ZIMMER czimmer@pasteur.fr	https://sites.google.com/site/imagingandmodeling/ https://research.pasteur.fr/en/team/imaging-and-modeling/
4	Genomes and Genetics	**Genetics of Macromolecular Interactions** (Alain JACQUIER)	Mechanisms of RNA degradation through nonsense-mediated mRNA decay (NMD) in eukaryotes	Cosmin SAVEANU cosmin.saveanu@pasteur.fr	https://research.pasteur.fr/fr/team/group-cosmin-saveanu/
5	Genomes and Genetics	**Mouse Genetics** (Xavier MONTAGUTELLI)	Identifying genes implicated in host response differences to Salmonella infection using new murine genetic reference populations	Jean JAUBERT jean.jaubert@pasteur.fr	https://research.pasteur.fr/fr/team/mouse-genetics/

6	Immunology	**Cytokine Signaling** (Sandra PELLEGRINI)	Modulation of the human T cell response by type I interferon	Frédérique MICHEL frederique.michel@pasteur.fr	https://research.pasteur.fr/fr/team/cytokine-signaling/ https://research.pasteur.fr/en/program_project/milieu-interieur-labex/
7	Structural Biology and Chemistry	**Structural Microbiology** (Pedro M. ALZARI)	The actinobacterial exception: structure and regulation of a central metabolic supercomplex	Marco BELLINZONI marco.bellinzoni@pasteur.fr	https://research.pasteur.fr/en/team/group-marco-bellinzoni/
8	Structural Biology and Chemistry	**Biochemistry of Molecular Interactions** (Daniel LADANT)	Membrane insertion and translocation of the CyaA toxin	Alexandre CHENAL alexandre.chenal@pasteur.fr	https://research.pasteur.fr/en/team/biochemistry-of-macromolecular-interactions/ https://research.pasteur.fr/en/team/group-alexandre-chenal/
9	Virology	**Structural Virology** (Félix A. REY)	Structural and functional characterization of intact pestivirus non-structural membrane protein 2 (NS2).	M. Alejandra TORTORICI/ Prof. Félix A. REY maria-alejandra.tortorici@pasteur.fr	https://research.pasteur.fr/fr/team/structural-virology/
10	Developmental & Stem Cell Biology	**Heart Morphogenesis** (Sigolène MEILHAC)	Left-right patterning of heart morphogenesis	Sigolène MEILHAC sigolene.meilhac@pasteur.fr	https://research.pasteur.fr/en/team/heart-morphogenesis/
11	Microbiology	***Yersinia* Research Unit** (Javier PIZARRO CERDA**)**	How can *Yersinia pestis* become invisible to the host immune system?	Christian DEMEURE cdemeure@pasteur.fr	https://research.pasteur.fr/fr/team/yersinia/

12	Center for Innovation and Technological Research & Cell Biology and Infection	**Photonic BioImaging (UtechS)** (Spencer SHORTE)	New tool for visualizing and detecting *Klebsiella pneumoniae* infections	Régis TOURNEBIZE regis.tournebize@pasteur.fr	https://research.pasteur.fr/en/team/photonic-bioimaging-utechs-pbi/

PROJECT 1

ROLE OF INTERMEDIATE FILAMENTS IN MECHANOTRANSDUCTION DURING CELL MIGRATION

Keywords: Migration, cytoskeleton, intermediate filaments, mechanotransduction, nuclear organization
Department: Biologie Cellulaire et Infection
Name of the lab: Cell polarity, migration and cancer lab.
Head of the lab: Sandrine Etienne-Manneville
Ph.D. advisor: Sandrine Etienne-Manneville
E-mail address: setienne@pasteur.fr
Web site address of the lab: etienne-manneville-lab.com
***Doctoral school affiliation and University*:** CdV, UPMC

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

Our research focuses on the control of cell polarity and cell migration in health and disease. Our aim is to decipher the molecular mechanisms controlling cell polarity, and to determine how polarized cell organization contribute cell migration. We tackle this question by studying astrocyte migration. Astrocytes are major glial cells of the central nervous system. Under pathological situations involving inflammation of the cerebral tissue, astrocytes become reactive, polarize and migrate in the direction of the inflammatory site. In these conditions, polarization and migration are tightly regulated. In case of cerebral injuries, astrocyte migration is a key parameter influencing the position of the glial scar and thus the region of axonal recovery.

Over the last years, we have used primary astrocytes in *in vitro* polarization and migration assays,

(i) to investigate the nature of the polarity cues. After demonstrating the importance of astrocyte interactions with the extracellular matrix and with neighboring cells, we have more recently determine how collectively migrating cells control the dynamics of cell-cell adhesions.

(ii) to characterize the role of evolutionary conserved polarity proteins, including Cdc42, Scrib, APC (Adenomatous Polyposis Coli) and Dlg1 (Discs Large), in the signaling cascades that control cell polarization. We have recently identified a new protein interaction domain (SADH domain) present in Scrib and controlling its dynamics at the cell cortex. We have also studied the specific role of 2 Cdc42 splice variants during glial cell migration.

(iii) to determine how polarity signaling cascades control the organization of the cytoskeletal networks composed of microtubules and intermediate filaments to promote astrocyte polarization and migration. We have made important progress in characterizing the mechanisms controlling intermediate filament turnover and polarization and we have two on-going studies on the role of microtubule post-translational modification in the control of cell polarity and cell migration.

In addition to their role in cerebral injuries, astrocytes or their progenitors can give rise to astrocytomas and glioblastomas, the most common primary brain tumors, and the second most frequent tumors in children. These tumors are associated with a very poor prognosis, mainly due to

their invasive properties allowing tumor cells to escape local therapies. We have now broaden the scope of our goals by investigating how molecular alterations of key regulators of astrocyte polarization during migration may affect the behavior of astrocyte derived tumor cells.

DESCRIPTION OF THE PROJECT:

The cell cytoskeleton is mainly composed of three distinct filamentous networks: actin microfilaments, microtubules and intermediate filaments (IFs). Until now, actin and microtubule functions have been extensively studied, but much less is known about the role of IFs. Several lines of evidence point to a role of IFs in cell mechanics and cell migration (Leduc and Etienne-Manneville, 2015). Changes in the composition and the network organization of IFs occur during cell migration and participate in tumor cell invasion. Using in vitro models of astrocyte and glioblastoma cell migration we have demonstrated that IFs control cell polarity and nucleus positioning (Dupin and Etienne-Manneville, 2011; Dupin et al., 2011). Cell interaction with the extracellular matrix triggers signaling cascades leading to IF rearrangements (Leduc & Etienne-Manneville, J Cell Biol 2017, in press). Our hypothesis is that the physical properties of the cell microenvironment change IF organization to change nuclear shape, orientation and position and to ultimately affect nuclear architecture and gene expression. The general goal of this project is to determine how IFs respond to the physical properties of the cell microenvironment to control nuclear positioning, rotation and shape and how IFs affect nuclear organization and gene expression.

The three major aims of this project are :

1 **To study how IF organization is affected by the physical properties of the substrate.**

Substrates of controlled rigidity and composition will be used to assess IF organization in cells plated on micropatterns to control cell shape, or in migrating cells. The involvement of mechanosensing proteins (Talin, vinculin) at focal adhesions will be tested.

2 **To characterize the interaction between IF and the nucleus**

Since the spatial organization of IFs around the nucleus is still poorly characterized, analysis of the nanoscale organization of IFs in proximity of the nuclear envelope will be done in order to identify key molecular linkers using 3D super-resolution imaging with an astigmatic dual color STORM (Herbert et al., 2012) to visualize IFs near the nuclear envelop and nesprin 3, a nuclear envelop protein interacting with IFs. The role of nesprins 3 and SUN proteins in the perinuclear organization of IFs and in IF role in nuclear positioning will be determined.

3 To determine if IFs influence nuclear organization and gene expression.

Depletion of one, two or three IFs proteins, of nesprin-3 and other nuclear IF linkers (identified in part 2) will done using siRNA and eventually gene editing. The characterization of the nuclear envelop, and the nuclear organization will be done using immunostaining (lamin, histone post-translational modifications…) and live cell imaging using cells expressing fluorescent markers of the centromeres. RNA-FISH will be used to follow the localization and activity of cytoskeletal genes (known to be modified in response to IF expression levels).

The three parts of project will show how IFs can transmit information from mechanotransduction sites, such as focal adhesions, to the nucleus to control gene expression in response to mechanical

stimulation. It will contribute to our understanding of IF functions in cell migration and tumor progression. This project will use multidisciplinary and innovative methods to bridge the gap between the in vitro molecular characterization and the physiopathological functions of IFs. This project will help us to underscore the role of IFs in cell mechanoresponses, a fundamental function for both developmental and cellular biology which currently receives a lot of attention.

REFERENCES:

Dupin, I., and Etienne-Manneville, S. (2011). Nuclear positioning: mechanisms and functions. Int J Biochem Cell Biol 43, 1698-1707.

Dupin, I., Sakamoto, Y., and Etienne-Manneville, S. (2011). Cytoplasmic intermediate filaments mediate actin-driven positioning of the nucleus. J Cell Sci 124, 865-872.

Herbert, S., Soares, H., Zimmer, C., and Henriques, R. (2012). Single-molecule localization super-resolution microscopy: deeper and faster. Microscopy and microanalysis : the official journal of Microscopy Society of America, Microbeam Analysis Society, Microscopical Society of Canada 18, 1419-1429.

Leduc, C., and Etienne-Manneville, S. (2015). Intermediate filaments in cell migration and invasion: the unusual suspects. Curr Opin Cell Biol 32, 102-112.

Leduc C, Etienne-Manneville, S. (2017) Regulation of microtubule-associated motors drives the polarization of the intermediate filament network. J. Cell Biol.;216(6):1689-1703.

EXPECTED PROFILE OF THE CANDIDATE:

We are looking for highly motivated student. Experience in either cell biology, microscopy, mechanobiology, chromatin modification or nuclear organization would be a plus.

PROJECT 2

FUNCTIONAL STUDY OF THE TIFA-DEPENDENT INNATE IMMUNE RESPONSE DURING *CHLAMYDIA TRACHOMATIS* INFECTION

Keywords: innate defense, signaling cascade, female genital tract, *Chlamydia trachomatis*, bacterial infection
Department: Cell Biology & Infection
Name of the lab: Cellular Biology of Microbial Infection
Head of the lab: Agathe Subtil
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Web site address of the lab: https://research.pasteur.fr/en/team/cellular-biology-of-microbial-infection/
***Doctoral school affiliation and University*:** Complexité du Vivant (ED515 Université Pierre et Marie Curie)

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

Our laboratory studies the interactions between 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 referred to inclusion. 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. In particular, we aim at better understanding how epithelial cells, the main target of *Chlamydia*, respond to *C. trachomatis* infection and the mechanisms by which the bacteria manipulate host defense to evade immune clearance.

DESCRIPTION OF THE PROJECT:

Chlamydia trachomatis is a particularly prevalent human pathogen responsible for loss of eyesight through trachoma and the most common sexually transmitted disease of bacterial origin. Most of the patients infected by *C. trachomatis* are asymptomatic and remain untreated, leading to chronic or repeated infection in the female genital tract with severe outcomes such as infertility and pelvic inflammatory disease. *C. trachomatis* develops exclusively within host cells, mainly epithelial cells, inside a membrane-bound compartment called the inclusion. Epithelial cells sense and respond to the infection, for instance with the secretion of cytokines and chemokines aiming at eradicating the bacteria. However, the signaling pathways involved, and to which extent the bacteria manipulate this host response, remain poorly understood. TNF-α receptor-associated factor (TRAF)-interacting protein with a forkhead-associated (FHA) domain (**TIFA**) is a molecule interacting with TRAF2 and TRAF6[(1,2)]. In human embryonic kidney (HEK) 293 cells, TIFA has been shown to promote oligomerization and ubiquitination of TRAF6, thereby activating IκB kinase (IKK) and inducing NF-κB

activation[3]. TIFA oligomerization mediated by intermolecular binding between TIFA-FHA domain and TIFA-phosphorylated threonine 9[4], has been shown to initiate the TRAF6 oligomerization upon TNF-α stimulation and upon infection with gram-negative bacteria in HEK293 and vascular endothelial cells[4-6]. TIFA was also recently reported to be required for the detection of heptose-1,7-bisphosphate (HBP), a newly discovered pathogen associated molecular pattern derived from gram-negative bacteria during lipopolysaccharide synthesis[5]. The HBP/TIFA axis contributes to IL8 production in response to the infection by enteroinvasive bacteria *S. flexneri* and *S. typhimurium* in intestinal epithelial cells[7]. While TIFA implication in the detection of *C. trachomatis* infection has not yet been characterized, our preliminary observations show that TIFA localizes at the periphery of the C*hlamydia* inclusion.

The present studies will focus on the innate immune response of the host upon *C. trachomatis* infection in the female genital tract. In particular, we will investigate the role of TIFA during *C. trachomatis* infection. Given that the stimulations/infections induce TIFA oligomerization and subsequent activation of NF-κB cascades[1-3], we will examine the expression and oligomerization of TIFA in human cervical epithelial Hela cells infected by *C. trachomatis*. Different TIFA constructs[3,4,7] including WT, mutation of threonine 9, FHA mutant KRN and TRAF6-binding-defective mutant E178A, will be used and the localization and oligomerization of TIFA will be determined by immunofluorescence and immunoblot using native gel electrophoresis, respectively. TRAF2 and TRAF6 localization and NF-κB activation will also be examined. The level of *C. trachomatis*-elicited inflammation in Hela cells expressing these constructs, or when TIFA expression is silenced by siRNA, will be measured. We will also examine the signaling pathways involved in TIFA activation during *C. trachomatis* infection. The results will be confirmed in primary epithelial cells isolated from the genital tract of female patients, following a protocol already established by the host laboratory. The potential importance of TIFA in *C. trachomatis* infection of the female genital tract will be examined in a mouse model of infection, using TIFA knock-out mice. The inflammation and bacterial burden in the local genital tract will be monitored. Finally, using pull-down and co-immunoprecipitation approaches, we will study the mechanism by which TIFA is recruited to the *C. trachomatis* vacuole, possibly by binding one of the numerous bacterial proteins inserted in this compartment.

Collectively, the project will permit to better understand the contribution of TIFA to the innate immune response upon *C. trachomatis* infection in epithelial cells, and to identify the mechanism of TIFA activation during this infectious process. This may help to develop the potential new strategies by interfering with TIFA signaling cascades to fight infection by *C. trachomatis* in the female genital tract. If we discover that *C. trachomatis* manipulates the HBP/TIFA signaling axis, and the underlying molecular mechanisms, this work could suggest novel strategies to modulate TIFA-induced inflammation in infectious contexts.

REFERENCES:

1. Kanamori, M. et al. T2BP, a novel TRAF2 binding protein, can activate NF-kappaB and AP-1 without TNF stimulation. *Biochem Biophys Res Commun* **290**, 1108-1113, doi:10.1006/bbrc.2001.6315 (2002).
2. Takatsuna, H. *et al.* Identification of TIFA as an adapter protein that links tumor necrosis factor receptor-associated factor 6 (TRAF6) to interleukin-1 (IL-1) receptor-associated kinase-1 (IRAK-1) in IL-1 receptor signaling. *J Biol Chem* **278**, 12144-12150, doi:10.1074/jbc.M300720200 (2003).

3. Ea, C. K. et al. TIFA activates IkappaB kinase (IKK) by promoting oligomerization and ubiquitination of TRAF6. *PNAS* **101**, 15318-15323, doi:10.1073/pnas.0404132101 (2004).
5 Gaudet, R. G. *et al.* INNATE IMMUNITY. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity. *Science* **348**, 1251-1255, doi:10.1126/science.aaa4921 (2015).
4. Huang, C. C. *et al.* Intermolecular binding between TIFA-FHA and TIFA-pT mediates tumor necrosis factor alpha stimulation and NF-kappaB activation. *Mol Cell Biol* **32**, 2664-2673, doi:10.1128/MCB.00438-12 (2012).
6. Lin, T. Y. *et al.* TIFA as a crucial mediator for NLRP3 inflammasome. *PNAS* **113**, 15078-15083, doi:10.1073/pnas.1618773114 (2016).
7. Milivojevic, M. et al. ALPK1 controls TIFA/TRAF6-dependent innate immunity against heptose-1,7-bisphosphate of gram-negative bacteria. *PLoS Pathog* **13**, e1006224 (2017).

EXPECTED PROFILE OF THE CANDIDATE:

The student will be highly motivated, hardworking, and with a good background in cell biology.

PROJECT 3

SUPER-RESOLUTION IMAGING AND COMPUTATIONAL MODELING OF DYNAMIC CHROMOSOME ARCHITECTURE

Keywords: Chromatin, polymers, simulations, super-resolution microscopy, nuclear architecture, DNA damage, gene expression
Department: Cell biology and infections
Name of the lab: Imaging and Modeling Unit
Head of the lab: Christophe Zimmer
PhD advisor: Christophe Zimmer
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Web site address of the lab: https://sites.google.com/site/imagingandmodeling/
https://research.pasteur.fr/en/team/imaging-and-modeling/
***Doctoral school affiliation and University*:** Complexité du Vivant, Frontiers in Life Sciences

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

The Imaging and Modeling Unit is an interdisciplinary team comprising experts in physics, optics, computer science, cell biology and microbiology. Our lab builds quantitative descriptions and models of chromosome architecture, and develops high-resolution and high throughput imaging approaches. Achievements of the lab include: (i) development of a computational imaging method to map nuclear gene territories in yeast, (ii) identification of determinants of chromosome positions, (iii) predictive modeling of dynamic chromosome organization, (iv) establishment of a non-invasive super-resolution imaging method, (v) development of a software to count individual mRNA in single cells, (vi) co-development of a new genome assembly method, (vii) characterization of mechanical chromatin properties in yeast, and (viii) investigation of how chromatin is altered during DNA damage. Current work focuses on the dynamic 3D architecture of chromosomes and its implications for cellular function, and on developing improved single molecule super-resolution imaging methods.

Keywords of lab interests:

super-resolution imaging, chromatin architecture, polymer simulations, virus-host interactions, deep learning, single molecule methods, expansion microscopy.

DESCRIPTION OF THE PROJECT:

The 3D architecture of chromosomes and its dynamics impacts all fundamental functions of the genome, from the regulation of gene expression to the preservation of genome integrity, but is not characterized and understood in detail. Although genome-wide chromosome conformation capture provides very rich data about population-averaged nuclear architecture features, it cannot provide direct views of chromosome structure in single cells and is restricted to fixed cells. Our laboratory develops experimental and computational imaging and modeling approaches to study the biophysical principles that underlay chromosome organization and some of its functional implications. Over the years, we have developed imaging based techniques to describe chromatin locus territories in yeast, and polymer simulations to predict chromosome configurations and movements in silico. We also develop super-resolution microscopy methods based on single molecule localization that achieve ~20-30 nm lateral resolution and that we have recently extended to 3D imaging of entire cells, to high throughput imaging of hundreds of cells, and to live cell imaging.

The proposed project aims to leverage our super-resolution imaging techniques in combination with several fluorescent labeling approaches to image yeast chromosomes at high resolution, with sufficient detail to allow computational tracing the chromosomal fiber in 3D throughout the nucleus, and to study how chromosomes change their folding properties and dynamics upon transcriptional activation or induced DNA double strand breaks. This project will build on recent experimental and computational work from our lab (in collaboration with E. Fabre) about the average properties of chromatin in yeast and how chromatin fiber properties are modified by DNA damage (Arbona et al. 2017, Herbert et al. 2017). It will also benefit from an ongoing (unpublished) study of chromatin fiber structure in human cells and on our experience with expansion microscopy, a powerful new super-resolution imaging technique based on inflation of the sample. The project will consist first in visualizing single chromosomes in isolation (but in situ), then all chromosomes together, at 3D resolutions well below 50 nm. This will allow direct comparison with our polymer simulations and enable iterative improvements of the computational model. We will then test experimentally induced alterations of nuclear architecture, by induction of DNA damage and gene expression. Furthermore, we will aim to extend the imaging approach to live cells. If successful, this project could provide the first direct high resolution views of chromosome architecture and dynamics in single cells and will shed new light on how this architecture affects or is affected by gene expression and DNA damage.

REFERENCES:

Chromatin stiffening underlies enhanced locus mobility after DNA damage in budding yeast.

S. Herbert, A. Brion, J.-M. Arbona, M. Lelek, A. Veillet, B. Lelandais, J. Parmar, F. Fernandez, E. Alamyrac, Y. Khalil, E. Birgy, E. Fabre, and C. Zimmer.

EMBO Journal. in press (2017).

Inferring the physical properties of yeast chromatin through Bayesian analysis of whole nucleus simulations.

Arbona JM, Herbert S, Fabre E, C Zimmer.

Genome Biology, 18:81, doi: 10.1186/s13059-017-1199-x (2017).

Computational models of large-scale genome architecture. Rosa A. & Zimmer C. *Int Rev Cell Mol Biol*, 307, 275-349 (2014).

Effect of nuclear architecture on the efficiency of double-strand break repair.
Agmon N, Liefshitz B, Zimmer C, Fabre E, Kupiec M.
Nature Cell Biology. Jun; 15(6): 694-9 (2013).

FISH-quant: automatic counting of transcripts in 3D FISH images.
Mueller F, Senecal A, Tantale K, Marie-Nelly H, Ly N, Collin O, Basyuk E, Bertrand E, Darzacq X, Zimmer C.
Nature Methods. Apr; 10(4): 277-8 (2013).

Superresolution imaging of HIV in infected cells with FlAsH-PALM.

Lelek M, Di Nunzio F, Henriques R, Charneau P, Arhel N, and Zimmer C.

Proc Nat Acad Sci USA, 109 (22): 8564-9 (2012).

QuickPALM: 3D real-time photoactivation nanoscopy image processing in ImageJ.

R. Henriques, M. Lelek, E. F. Fornasiero, F. Valtorta, C. Zimmer & M. M. Mhlanga
Nature Methods, 7, 339 - 340 (2010).

EXPECTED PROFILE OF THE CANDIDATE:

We are open to candidates from different backgrounds, including but not limited to cell biology, molecular biology, physics, and computer science. We expect high dedication, autonomy and ability to work in a highly interdisciplinary environment.

PROJECT 4

MECHANISMS OF RNA DEGRADATION THROUGH NONSENSE-MEDIATED MRNA DECAY (NMD) IN EUKARYOTES

Keywords: RNA degradation, mRNA translation, eukaryotes, NMD, protein complexes
Department: Genomes and Genetics
Name of the lab: Genetics of Macromolecular Interactions
Head of the lab: Alain Jacquier
PhD advisor: Cosmin Saveanu
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Web site address of the lab: https://research.pasteur.fr/en/team/group-cosmin-saveanu/
***Doctoral school affiliation and University*:**

SHORT ABSTRACT

NMD is a major degradation pathway affecting cytoplasmic RNAs in eukaryotes. Our recent discovery of two distinct NMD complexes in yeast identified a new NMD-related degradation pathway. The project aims to explore these new data and build a unified model of NMD mechanisms in eukaryotes. The majority of the experimental work will be done in yeast (*Saccharomyces cerevisiae*). Large-scale proteomics and RNA sequencing experiments will be combined with targeted protein labeling and gene alterations including deletions and point mutations. The project involves both bench work and the corresponding data analysis tasks (RNASeq and mass spectrometry results).

PhD PROJECT DESCRIPTION

Context:
NMD, **non-sense mediated mRNA decay**, is a major degradation pathway for cytoplasmic mRNAs that contain premature termination codons. The pathway is conserved in eukaryotes and essential in *D. melanogaster*, zebrafish and mouse. It affects thousands of RNAs that can represent a sizeable fraction of the transcriptome as recently shown in our laboratory (Malabat, Feuerbach, Ma, Saveanu & Jacquier, *eLife* 2015). Most of these substrates have short open reading frames (Decourty, …, Saveanu, *Cell Reports* 2014) followed by long 3' untranslated regions (Amrani et al., *Nature* 2004). Despite the fact that defects in NMD affect telomerase activity, embryon development, translation termination and expression of oncogenes, the complexity of the process did not allow, until now, a clear view of the involved molecular mechanisms, even if the key factors were known for about 15 years.
NMD substrates are recognized through translation and rapidly degraded in a process that depends on three highly conserved factors, **Upf1**, an ATP dependent RNA helicase, **Upf2** and **Upf3**. The most detailed molecular mechanism described to date (reviewed in Karousis, Nasif & Muhlemann, *WIREs RNA* 2016) involves the phosphorylation of the major NMD factor Upf1 by a protein kinase in proximity of ribosomes blocked on an mRNA due to non-optimal translation termination conditions. Phosphorylated residues assist the recruitment of other factors that can either directly cleave the RNA (**Smg-6**) or recruit RNA degradation factors (**Smg-5/7** heterodimer). Dephosphorylation of Upf1 is thought to allow recycling of the protein for a new cycle of NMD. Recent data indicate that **alternative** molecular mechanisms could be involved in NMD. For example, Smg6 was shown to interact with Upf1 in the absence of phosphorylation and several previously identified interactions

between NMD factors could not be detected in large-scale quantitative proteomic studies (Huttlin et al *Nature* 2017, Hein et al., *Cell* 2015). The phosphorylated region of Upf1 is not conserved in unicellular eukaryotes, despite the fact that NMD is active in these organisms.

By using quantitative mass-spectrometry on affinity purified NMD complexes we obtained the most extensive characterization of NMD complexes in a eukaryote (Dehecq et al., manuscript in preparation). We are working with the yeast *Saccharomyces cerevisiae*, an excellent experimental system for NMD. Our results, based on more than 100 experiments with 16 strains under 29 different experimental conditions identified two new, succesive NMD complexes and uncovered a wealth of new questions about the involved molecular mechanisms. These discoveries, together with observations made on RNASeq data in NMD mutants represent the bases for the present PhD project.

Aims and strategy:

Our unpublished results, based on affinity purification of NMD complexes and quantitative mass spectrometry, identified two mutually exclusive NMD complexes in yeast. Why these complexes are formed, how do they participate to the recognition of RNA substrates and their degradation, and to what extent similar NMD complexes exist in other organisms is currently unknown.

Aim 1: Elucidate the role of the two newly described NMD complexes. The PhD candidate will generate Upf1 mutants and screen for those showing an alteration in their ability to participate to each of the two identified complexes. Testing RNA substrates for the efficiency of NMD in mutated strains will allow pinpointing the specific role of different Upf1 forms. Affinity purification of different Upf1 isoforms and of other components of the NMD pathway followed by RNASeq will provide a panorama of the RNAs associated with the complexes and should give hints about the recruitment of Upf1 on RNA and the events that are responsible.

Aim 2: Investigate alternative degradation mechanisms for NMD substrates. Our unpublished data indicate that NMD complexes contain a potential RNA endonuclease. The student will investigate the specific potential roles of this endonuclease, either as an enzyme or as a co-factor for the Upf1 ATPase on NMD targets by testing the association of the protein and of a catalytically inactive version of it to RNA genome-wide. This tests will be done both with a classical affinity-purification followed by RNA sequencing approach and with a new method involving the fusion of the protein of interest with a poly(U) polymerase (Lapointe et al., *Nature Methods* 2015). To test "*in vivo*" the role of this endonuclease in NMD, RNA sequencing will be performed with strains lacking the enzyme and other major factors involved in RNA degradation (both 5' to 3' and 3' to 5' exonucleolysis).

Aim 3: Understand how RNA decapping is triggered in NMD. Current biochemical models of mammalian NMD invoke the role of the Smg-1 protein kinase in the activation of Upf1. We identified an essential protein kinase in NMD RNA-protein complexes in yeast that we believe to play a regulatory role during the first step in the degradation of NMD substrates, RNA decapping. Point mutations and rapid depletion of this essential kinase will be used to investigate this hypothesis.

Significance: The main aim of this project is to provide new insights into NMD mechanisms conserved from yeast to humans. The study of NMD is intimately linked with the study of translation, mRNA stability and the diversity and “fuzziness” of DNA transcription. Knowledge of these processes is essential to understand gene expression.

RECENT PUBLICATIONS (SUPERVISOR AND TEAM MEMBERS UNDERLINED):

Babiano R, Badis G, Saveanu C, Namane A, Doyen A, Díaz-Quintana A, Jacquier A, Fromont-Racine M & de la Cruz J (**2013**) Yeast ribosomal protein L7 and its homologue Rlp7 are simultaneously present at distinct sites on pre-60S ribosomal particles. *Nucl. Acids Res*. 41: 9461-9470. PMID: 23945946

Defenouillère Q, Yao Y, Mouaikel J, Namane A, Galopier A, Decourty L, Doyen A, Malabat C, Saveanu C, Jacquier A & Fromont-Racine M (**2013**) Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. *Proc. Natl. Acad. Sci. U.S.A*. 110: 5046–5051. PMID: 23479637

Decourty L, Doyen A, Malabat C, Frachon E, Rispal D, Séraphin B, Feuerbach F, Jacquier A & Saveanu C (**2014**) Long open reading frame transcripts escape nonsense-mediated mRNA decay in yeast. *Cell Rep* 6: 593–598. PMID: 24529707

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Reviews and book chapters:

Fromont-Racine M & Saveanu C (**2014**) mRNA Degradation and Decay. book chapter in *Fungal RNA Biology* : 159–193 (Springer, edited by Ane Sesma and Tobias von der Haar). ISBN: 978-3-319-05686-9

Malabat C & Saveanu C (**2016**) Identification of Links Between Cellular Pathways by Genetic Interaction Mapping (GIM). book chapter in *Methods Mol Biol.* 1361:325-43, Yeast Functional Genomics (Springer, edited by Frédéric Devaux). PMID: 26483030 ISBN: 978-1-4939-3078-4

Saveanu C & Jacquier A (**2016**) How cells kill a 'killer' messenger. *Elife* 5 pii: e16076 (insight). PMID: 27120800

PROJECT 5

IDENTIFYING GENES IMPLICATED IN HOST RESPONSE DIFFERENCES TO SALMONELLA INFECTION USING NEW MURINE GENETIC REFERENCE POPULATIONS

Keywords: Salmonella, QTL, Genetic reference population, CC, DO
Department: Genomes & Genetics
Name of the lab: Mouse Genetics
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PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

The identification of host genetic variants associated with increased or decreased resistance to infection reveals critical mechanisms in the complex interplay between pathogens and their host, and is instrumental to the development of effective therapies. The Mouse Genetics laboratory focuses on identifying new genes and mechanisms involved in host immune response differences to diverse pathogens: i) bacteria, such as Yersinia or Salmonella; ii) viruses, such as Zika, Rift Valley Fever and West Nile. We are using the mouse model and newly developed murine genetic reference population (GRP) such as the Collaborative Cross (CC) or the Diversity Outbred (DO) to perform quantitative trait locus (QTL) mapping. Once candidate genomic regions and/or new strains with contrasted resistance or susceptibility phenotypes have been identified, we search for the causative genes using a mixture of genetic and pathophysiology approaches.

DESCRIPTION OF THE PROJECT:

Salmonella is a Gram-negative bacterium responsible for typhoid fever and diarrheic disease. It is one of the leading causes of food-borne infections and remains a major threat for human population [1–3]. Non-typhoidal *Salmonella* (NTS) serovars infect both humans and animals, cause a significant disease burden, with an estimated 93.8 million human cases and 155,000 deaths worldwide each year for *Salmonella* Typhimurium [4]. The variable outcome of Salmonella infections depends on many parameters, including the bacterial strain, environmental factors and host genetic makeup [5].

Infection of mice with *Salmonella* Typhimurium is widely used as an experimental model of human typhoid fever [6]. In infected mice, there is rapid localization and replication of the bacterium in the spleen and the liver. Laboratory mouse strains display a wide range of susceptibilities [7, 8]. Significant advances in understanding the host response to *Salmonella* Typhimurium infection have

been made over the years with the identification of genes such as *Slc11a11* (*Nramp1*), *Tlr4* and *Btk*, first in the mouse model [9–11] and later in humans and other animal species.

Quantitative trait loci (QTLs) mapping based on two generations crosses from parental strains differing in their susceptibility usually results in identifying broad confidence intervals and identifying the causative gene(s) can be very challenging [12]. To overcome this problem, a large panel of new inbred mouse strains, namely the Collaborative Cross (CC), was developed over the last decade through a global community effort [13]. CC strains are recombinant inbred strains derived from eight distinct founder strains that include five classical laboratory strains combined with three wild-derived strains [14, 15]. CC strains represent a genetically heterogeneous population with an even distribution of allelic variation, which closely resembles the genetic diversity found in human population.

We recently used the CC mouse population to identify new regions involved in the complex host response to *Salmonella* Typhimurium infection. We mapped two significant and one suggestive QTLs associated with spleen bacterial burden differences, and identified candidate genes within each QTL interval. We also identified four CC strains with extreme phenotypes: one extremely susceptible and three more resistant than classical laboratory strains.

The proposed thesis project would consist in pursuing forward functional genetics to identify the causative genes underlying these newly identified QTL regions, and in unravelling mechanisms at work in CC strains exhibiting extreme phenotypes. We are also studying susceptibility to *Salmonella* Enteritidis infection as a model of chronic carriage. Preliminary QTL mapping results suggest that there is likely one common region implicated in host response differences in both acute and chronic infection models.

The functional genetics approach will consist in using another murine GRP resource recently created, namely the Diversity outbred (DO) population, which was derived from the same eight parental founder strains as the CC. It has accumulated recombination events and has therefore a higher mapping resolution [16]. Combining mapping data from CC and DO mice will allow reducing the number of candidate genes. Likewise, we will combine data from *S.* Typhymurium and *S.* Enteritidis models in common QTL region and use genomic sequence data from the 8 founders and variant prediction analysis tools to select the best candidate genes. These candidates will undergo functional validation to confirm their causality. This will be performed *in vitro* by comparing infection response to *Salmonella* of standard MEF cells and MEF cells in which candidate genes have inactivated by CRISPR/Cas9. *In vivo* analysis by complementation crosses will be performed depending on knock-out mouse model availability for each gene. In parallel detailed phenotypic and immunological characterization of the CC strains with extreme phenotypes will be performed.

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PROJECT 6

MODULATION OF THE HUMAN T CELL RESPONSE BY TYPE I INTERFERON

Keywords: human immunology, autoimmunity, inflammation, gene regulation
Department: Immunology
Name of the lab: Unit of Cytokine Signaling, INSERM U1221
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***Doctoral school affiliation and University*:** ED 394 Physiology, Physiopathology and Therapeutic, UPMC University

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

Work in the Unit of Cytokine Signaling aims at deciphering molecular mechanisms that govern protective and pathogenic effects of type I interferons in the human immune system. Our research topics are mainly focused on the IFN signaling pathway and its regulation, the functional impact of polymorphism of genes associated with inflammatory diseases, and the immunomodulatory role of IFN-I.

DESCRIPTION OF THE PROJECT:

The type I IFN family (IFN-α/β) exerts a complex immunomodulatory activity which can be beneficial or deleterious according to the pathophysiological context. The project aims at understanding the 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. The relapsing-remitting form of this chronic neuroinflammatory and autoimmune disease is commonly treated by IFN-β.
One objective is to pursue our study on the molecular mechanisms by which IFN promotes the expression of the anti-inflammatory cytokine IL-10 in $CD4^+$ T cells stimulated through the T cell receptor (TCR). Using large scale transcriptomic and RNAi approaches, we have identified some transcription factors and STAT family members that are involved in the IFN-dependent enhancement of IL-10 expression. Mechanistic insights into the activation and the transcriptional role of these factors will be gained through studies of the TCR and IFN signaling pathways, chromatin immunoprecipitation and RNAi assays, using primary $CD4^+$ T cells and $CD4^+$ T cell lines. A second objective is to characterize IFN-induced type 1 regulatory-like cells (Tr1-like cells). Based on our RNA-seq data, a molecular signature of these cells will have to be validated. To this end, multiplex qPCR will be set up in bulk and at the single cell level. The functional activity and stability of IFN-Tr1 cells will be also investigated. Insights from this project with healthy donors will be translated to multiple sclerosis patients, taking advantage of the translational project that we are developing.

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EXPECTED PROFILE OF THE CANDIDATE:

Skills in transcriptome analysis, regulation of gene expression, bioinformatics and cellular immunology are expected

PROJECT 7

THE ACTINOBACTERIAL EXCEPTION: STRUCTURE AND REGULATION OF A CENTRAL METABOLIC SUPERCOMPLEX

Keywords: Actinobacteria; tuberculosis; Corynebacterium; integrative structural biology; macromolecular complexes; metabolism; X-ray crystallography; cryo-EM
Department: Structural Biology and Chemistry
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***Doctoral school affiliation and University*:** MTCI (ED436) / Paris Diderot (Paris 7)

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 bacterial biochemistry and structural biology. Although spanning over several projects, the lab's activity has been focused on the elucidation of the molecular basis of signal transduction in bacteria, with notable achievements on the structure and function of Ser/Thr kinases [1-3], phosphatases [4,5] and two-component systems [6-8]. The lab is now organized in four groups led, respectively, by Pedro M. Alzari (Unit leader), Claudine Mayer, Jean-Christophe Barale and Marco Bellinzoni, in which the PhD student will be enrolled. The group is dedicated to the study of structure and regulation of macromolecular complexes in actinobacteria and has contributed to show, through a wide European collaboration, how mycobacteria can tune their central metabolism according to the available carbon and nitrogen sources [9-11]. A strong multidisciplinary approach that includes microbiology, biochemistry and structural biology is the common theme in the Unit and stays at the heart of our research activities. The Unit has several, well-established collaborations both at the national and international level, including groups in Europe (Germany, UK, Italy), South America (Argentina, Brazil, Uruguay) and Asia (Cambodia). Elucidating the molecular basis of key biological processes to develop new therapeutic strategies is our common goal.

DESCRIPTION OF THE PROJECT:

Actinobacteria represent one of the largest eubacterial taxonomic groups and include important human pathogens like *Mycobacterium tuberculosis*, invaluable sources of antibiotics and natural compounds like *Streptomyces*, and major industrial cell factories like *Corynebacterium glutamicum* [12]. The need to improve our knowledge of the actinobacterial molecular physiology has been increasing, either for therapeutics (e.g. development of new antibiotics), or for biotechnological purposes, *i.e.*

the optimization of strains for the industrial production of chemicals that range from amino acids to terpenes and alcohols. In our lab, we uncovered unique features in the way some of the most conserved reactions in central metabolism are carried out and regulated in *Mycobacterium,* especially the ones situated at two of the most central metabolic nodes, pyruvate and a-ketoglutarate [11,13]. Despite being considered as universally conserved, we showed that two of the most important complexes, i.e. pyruvate dehydrogenase (PDH) and oxoglutarate dehydrogenase (ODH), are actually merged together in these bacteria to make a mixed 'supercomplex'. Our recent data suggest this supercomplex to represent a trait of the Actinobacteria phylum. Such findings not only have focused our interest as structural biologists, willing to solve the 3D structure of such a fascinating enzymatic machine, but raise a lot of questions about how this supercomplex is regulated, in a way to coordinate key reactions and metabolic fluxes at two crucial nodes, where carbon metabolism and nitrogen assimilation diverge.

The PhD student will be enrolled in our ongoing work, whose first goal is to isolate this complex and/or reconstitute it *in vitro*, in order to characterize it by an integrative structural biology approach. In practice, daily work will involve different methodologies carried out through high-end equipment, either on the institute's campus or outside, including X-ray crystallography, SAXS (Small Angle X-ray scattering), mass spectrometry and cryo-electron microscopy, in close collaboration with the cutting-edge platforms at Pasteur. We already determined the crystal structures of the four enzymes forming the supercomplex, and have succeeded in coexpressing them together in *E. coli*, opening the way to the structural characterization of the whole complex. The student will therefore have the opportunity to learn and apply a number of techniques to determine this exciting structure and see how the complex functions, alternating wet-lab with computational work. In addition, through a recently established collaboration with the groups of Profs. Michael Bott and Bernhard Eikmanns, both located in Germany, we wish to go beyond a static structural picture, identifying the regulators that act to coordinate these reactions. *C. glutamicum* will be our working organism, given that this species is a well-established lab model and a major tool for the biotechnological industry [14]. The final goal is to clarify 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 both for drug development (*e.g.* by targeting essential protein-protein interactions), and for the improvement of industrially relevant strains. Indeed, the metabolic checkpoints we are looking at are hotspots for metabolic engineering [14].

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EXPECTED PROFILE OF THE CANDIDATE:

The ideal PhD candidate for this project is a brilliant and enthusiastic master-level student, interested in microbiology as well as protein biochemistry and structural biology, and willing to learn a portfolio of complementary techniques. Solid communication and presenting skills, as well as a good sense of teamwork are essential. Documented experience in protein biochemistry, biophysics or structural biology would be an asset.

PROJECT 8

MEMBRANE INSERTION AND TRANSLOCATION OF THE CyaA TOXIN

Keywords: protein membrane interaction, protein membrane translocation, lipid bilayer, biophysics, fluorescence, FRET, biochemistry
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***Doctoral school affiliation and University*:** BioSPC

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.

The CyaA toxin is a major virulent factor produced by *Bordetella pertussis*, the causative agent of whooping cough. Its translocation process in target cells remains, however, largely unknown. 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 CyaA-based biotechnological applications developed in the lab, i.e., to improve the use of CyaA as 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 available in the Unit, in the Institut Pasteur Technological Centers and thanks to national and international collaborations.

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: the ATP-cyclizing, CaM-activated, catalytic domain (ACD) is located in the 364 amino-proximal residues. The region spanning residues 400 to 500 is involved in the translocation process of ACD while the C-terminal part of the molecule (from 500 to 1706) is involved in membrane insertion and toxin binding to a specific cellular receptor (CD11b/CD18). The CyaA toxin is synthesized as an inactive precursor, pro-CyaA, that is converted into the active toxin upon specific acylation of two lysine residues (Lys 860 and Lys 983). 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.

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 to monitor the translocation process developed in the lab will be used. 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

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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 9

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF INTACT PESTIVIRUS NON-STRUCTURAL MEMBRANE PROTEIN 2 (NS2)

Keywords: pestivirus, uncleaved NS2-NS3, membrane protein expression and purification, x-ray crystallography, single-particle cryo-electron microscopy (cryo-EM).
Department: Virology
Name of the lab: Structural Virology
Head of the lab: Prof. Dr. Félix A.Rey
Ph.D. advisor: M. Alejandra Tortorici/ Prof. Félix A. Rey
E-mail address: maria-alejandra.tortorici@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/fr/team/structural-virology/
***Doctoral school affiliation and University*:** Ecole Doctorale MTCI (Médicament-Toxicologie-Chimie-Imageries). Université Paris-Descartes.

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

Two of the main scientific goals of our research are:

1) to provide a structural basis for understanding the molecular mechanisms of membrane fusion used by enveloped viruses to enter into a target cell, and

2) to understand the molecular mechanism used by RNA viruses to replicate their genome by both structural and functional studies of viral RNA-dependent RNA polymerases (RdRp) and associated replication enzymes.

We study mostly viruses of global public health and/or of veterinary concern. The knowledge gained can be used for translational structure-based design of preventive or curative antiviral agents. Furthermore, these structural studies often provide crucial information about evolutionary relations between apparently unrelated viruses.

Our initial studies of the three-dimensional (3D) structure of the flavivirus and of the alphavirus envelope proteins introduced the concept of class II viral fusion proteins, which are formed by three globular domains -essentially constituted by b-sheets- arranged in a rod-like shape as opposed to the first described class I viral fusion proteins, which are characterized by a central trimeric a-helical coilded coil. We have also described the structures of rubi- and bunya-viruses envelope proteins, also belonging to the class II fusion proteins and more recently, we described the first structure of the class I coronavirus spike ectodomain in its pre-fusion form, determined using cryo-EM.

In addition, we have structurally and functionally characterized non-structural proteins such as the RdRp of the hepatitis C virus and more recently the pestivirus NS3 protease/helicase in complex with its cofactor, and enzyme that is also essential for viral genome replication and virus morphogenesis.

DESCRIPTION OF THE PROJECT:

Introduction: Pestiviruses infect a wide range of cloven-hoofed animals, wild and domestic, causing serious disease. The most studied are the classical swine fever virus (CSFV) (1) and the bovine viral diarrhea virus, which impose important economic losses to the livestock industry worldwide (2). Together with the closely related human hepatitis C virus (HCV) and flaviviruses like dengue or Zika viruses, pestiviruses belong to the *Flaviviridae* family of positive-sense RNA viruses. Their genome codes for a single polyprotein, which is processed by cellular and viral proteases to generate the individual mature viral proteins. The first proteolytic cleavage in the non-structural region is done by NS2, a 460 residues multi-pass trans-membrane cysteine protease. The remaining cleavages are done by NS3, which also has helicase activity. Both, NS2 and NS3 are essential for genome replication but also for virus morphogenesis. For pestiviruses, and in contrast to HCV, the cleavage between NS2 and NS3 in the polyprotein precursor is necessary for genome replication but abolishes particle morphogenesis. Upon infection, a host factor named Jiv, necessary for cleavage between NS2 and NS3, is available in the cytoplasm, but as replication ensues, Jiv becomes limiting and later rounds of polyprotein production lead to absence of cleavage thereby allowing virus morphogenesis.

State-of-the-art: This project follows our recent functional and structural characterization of the pestivirus CSFV NS3 (3, 4). By using structure-guided mutagenesis we have identified that NS4A appears to bind at a surface of NS3 that is occupied by NS2 in the uncleaved form (4). Thus, we identified a dual effect: while the NS2-NS3 junction is cleaved, NS4A can displace NS2 from the complex, and replication ensues. When NS3 is covalently linked to NS2, the NS4A factor, necessary for replication cannot bind, so replication cannot take place. This elegant control system, explains how pestiviruses temporally orchestrate the switch from genome replication to virion morphogenesis. Central to further understanding this process is determining the structure of its components, the main one missing being NS2, which has eluded structural studies so far (only its soluble cytosolic domain is known for HCV (5)).

Goals: 1. To determine the 3D-structure of the multi-pass trans-membrane NS2 protein. We have already identified promising conditions for protein production and detergent solubilization compatible with crystallization in collaboration with Nicolas Reyes on campus, who is an expert in membrane protein production for crystallization.

2. To understand the organization of the uncleaved NS2-NS3 protein on membranes, we are also preparing the ground to use cutting-edge single-particle cryo-EM to pursue this study, in combination with lipid nanodisc technology (6). The structural information obtained is expected to lead to the discovery of new strategies for antiviral research.

Step-wise research plan: 1) Optimization of constructs to produce high amounts of full-length CSFV NS2 in mammalian cells to be solubilized and inserted into nanodiscs to reconstitute the protein in its native environment. 2) Crystallization of CSFV NS2 using standard methods for membrane protein crystallization such as lipid cubic phase. 3) Determination of the 3D-structure of CSFV NS2 by X-ray crystallography or cryo-EM. 4) Development of functional studies to validate our structural data.

OPTIONAL PROJECT:

We recently described the first structure of the class I coronavirus (CoV) spike (S) ectodomain in its pre-fusion form, determined using cryo-EM (7). There is an increasing evidence, however, that the transmembrane and the cytosolic regions of class I proteins modulate the antigenicity of the exposed ectodomain as shown for HIV-1 envelope trimer (8). Therefore, it is crucial to understand the conformation of these regions in order to identify conformational epitopes to be targeted for efficient vaccine design. Thus, our goal is to determine the 3D-structure of a CoV full-length S protein embedded in a native bilayer environment by cryo-EM combined with lipid nanodisc technology (6).

NOTE: Both projects have a similar technical approach so the choice of the final project will depend on the particular interests of the selected student.

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3. Tortorici MA, Duquerroy S, Kwok J, Vonrhein C, Perez J, Lamp B, Bricogne G, Rumenapf T, Vachette P, Rey FA. 2015. X-ray structure of the pestivirus NS3 helicase and its conformation in solution. J Virol 89:4356-4371.

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8. Dev J, Park D, Fu Q, Chen J, Ha HJ, Ghantous F, Herrmann T, Chang W, Liu Z, Frey G, Seaman MS, Chen B, Chou JJ. 2016. Structural basis for membrane anchoring of HIV-1 envelope spike. Science 353:172-175.

EXPECTED PROFILE OF THE CANDIDATE:

Applicants should have a high degree of motivation and a willingness to work in a very competitive field. A previous experience with DNA techniques (PCR, cloning, agarose gels, plasmid DNA

purification etc), cell culture and protein expression and purification techniques (affinity columns, gel filtration, protein gels, etc) would be also greatly appreciated.

PROJECT 10

LEFT-RIGHT PATTERNING OF HEART MORPHOGENESIS

Keywords: : left-right patterning, asymmetric morphogenesis, congenital heart defects, mouse genetics, transcriptomics
Department: Developmental & Stem Cell Biology
Name of the lab: Heart Morphogenesis
Head of the lab: Sigolène Meilhac
Ph.D. advisor: Sigolène Meilhac
E-mail address: sigolene.meilhac@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/en/team/heart-morphogenesis/
***Doctoral school affiliation and University*:** ED562 BioSPC University Paris Descartes 5

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 drive asymmetric 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.

DESCRIPTION OF THE PROJECT:

The mammalian heart has four cardiac chambers, two atria and two ventricles. The alignment of cardiac chambers is key for the correct plumbing of the blood, so that carbonated blood in the right heart is separated from oxygenated blood in the left heart. Initially, in the heart primordium, the

right ventricle is positioned cranially to the left ventricle. It is during the process referred to as cardiac looping, that the right ventricle acquires its position to the right of the left ventricle. Cardiac looping corresponds to a rapid change in heart geometry, from a straight tube to a helical tube [see 6]. Heart looping, which is oriented rightward, is the first morphological sign of left-right asymmetry during embryo development. It has been shown to depend on left-right patterning, initiated one day earlier in the left-right organiser of the embryo [7]. In humans, mutations disrupting the left-right organiser are associated with heterotaxy, including defects in visceral organs and also complex cardiac malformations. However, how information from the left-right organiser is transposed into cardiac cells remains poorly understood.

The laboratory has recently developed a computer model of heart looping, as well as tools to stage heart looping and quantify in 3D the associated morphological changes. These are applied to study in mouse mutants the role of the major left determinant Nodal, a secreted factor of the TGFβ family, in heart looping.

As other factors than Nodal have been proposed to control heart looping in the fish [8], the PhD project aims at characterising novel markers of left-right asymmetry during heart looping. Which markers are asymmetrically expressed at precise stages of heart looping ? Are they dependent on Nodal signalling ? What is the proportion of cardiac cells that have expressed Nodal on the left side ? Molecular markers will be identified by transcriptomics, in control and Nodal mutant embryos. Transgenic mouse models will be developed to track cells which have expressed an asymmetric marker, including Nodal.

The project is expected to provide novel insight into how heart morphogenesis is imprinted by left-right signalling, which is relevant to congenital heart defects in humans.

REFERENCES:

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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 11

HOW CAN *Yersinia pestis* BECOME INVISIBLE TO THE HOST IMMUNE SYSTEM?

Keywords: Plague, Yersinia pestis, host cellular immune response, immune evasion
Department: Microbiology
Name of the lab: *Yersinia* Research Unit.
Head of the lab: Dr Javier Pizarro Cerda
Ph.D. advisor: Dr Christian Demeure
E-mail address: cdemeure@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/fr/team/yersinia/
***Doctoral school affiliation and University*:** Université Paris BioSPC

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

The activities of the *Yersinia* Research Unit are primarily devoted to the analysis of:
- Mechanisms of horizontal gene transfer in *Yersinia*.
- Comparative genomics and transcriptomics between *Y. pestis* and *Y. pseudotuberculosis*.
- Molecular bases for the exceptional pathogenicity of *Y. pestis*.
- Pathophysiology of *Yersinia* infections.
- Host's mechanisms of innate and adaptive immunity.
- Genetic bases of host susceptibility to plague.
- Resistance of pathogenic *Yersinia* to antibiotics.
- Evolution of pathogenic *Yersinia*.

The Unit is also developing:
- A vaccine against plague and pseudotuberculosis.
- Typing tools for molecular epidemiology.
- Real time *in vivo* imaging technologies for pathogenic *Yersinia*.
- Tools for stable gene complementation and gene expression *in vitro* and *in vivo*.
- Techniques for molecular characterization of the various *Yersinia* species.

The Unit participates actively to the surveillance and control of enteropathogenic *Yersinia* through its activities at the National level (Reference Laboratory and French Surveillance Network), and to the fight against plague at the international level (World Health Organization Collaborating Center for *Yersinia*).

DESCRIPTION OF THE PROJECT:

Y. pestis is the agent of plague, a disease transmitted from rodents to humans by fleabites. Bubonic plague is fatal for 50-70% of patients in the absence of treatment. Pneumonic plague results from inter-human contamination through aerosols and is an acute and fulminant pneumopathy, which is systematically lethal in usually less than 3 days. *Y. pestis* is thus among the most pathogenic bacteria

for humans. Despite considerable progress in plague prevention and cure, this infection has not been eradicated and natural plague foci exist in Africa, Asia and the Americas.

The key to *Y. pestis* virulence is its capacity to escape host immunity. During the first 48h of infection, the host's innate immune response is defective (pre-inflammatory phase), allowing the bacteria to multiply and invade tissues. How *Y. pestis* prevents this response and what are the bacterial virulence factors responsible for this inhibition remain open questions. The proposed project will aim at deciphering the bacteria-cell and cell-cell interactions that occur early during the infectious process to paralyze the host defense system.

The normal immune response against bacteria involves a cascade of signals starting from sentinel cells (dendritic cells (DC), macrophages), and continuing with the activation of other cell types such as natural killer cells (NK) and T lymphocytes to produce cytokines involved in effector mechanisms. In this project, NK and DCs recruitment to infected lymph nodes will be characterized using the mouse experimental model of bubonic plague. The dynamics of recruitment and expansion or destruction of these populations in the lymph node infected with *Y. pestis* will be analyzed (flow cytometry and fluorescence microscopy). Production of cytokines and chemokines activating/attracting NK cells will be examined. Among them, IFNγ will be particularly studied because the production of this cytokine that activates essential macrophage bactericidal functions is known to be severely impaired during plague. Immunohistology will be used to define cell localization and cell-cell contacts in the lymph nodes. The presence and source of NK-inactivating factors will be determined (Immunoassays/FISH). KO or transgenic mice will be used to confirm the roles played by the identified cell populations. Once specific specific cells and factors targeted early by *Y. pestis* are identified, the interactions between these targets and the bacteria will be deciphered using in vitro cultures of single and mixed cell populations. Cell survival, mechanisms of cell death, levels of cytokine production and activation markers will be examined.

Since the capacity to prevent the triggering of an early innate immune response is specific to the plague bacillus, another part of the project will aim at identifying the bacterial factors responsible for this unique property of *Y. pestis.* Two approaches will be used. One will benefit from the very close genetic relationship between *Y. pestis* and its ancestor *Y. pseudotuberculosis* (a much less virulent enteropathogen) to identify mechanisms of immune response inhibition that are triggered by *Y. pestis* but not *Y. pseudotuberculosis*. The second approach will consist in using a set of *Y. pestis* mutants devoid of various genetic elements acquired by *Y. pestis* after its divergence from *Y. pseudotuberculosis* (already available in the laboratory) to determine which factors play a role in the observed phenotypes. If necessary, additional mutants will be constructed.

This study should provide unprecedented understanding on how a highly pathogenic bacterium circumvents innate host defense mechanisms to invade and kill its host extremely efficiently.

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M., Balloux, F., Keim, P., Wirth, T., Ravel, J., Yang, R., Carniel, E., and Achtman, M. (2010) *Yersinia pestis* genome sequencing identifies patterns of global phylogenetic diversity. ***Nature Genetics***. **42**, 1140-1143

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EXPECTED PROFILE OF THE CANDIDATE:

The project is proposed for a PhD or post-Doc. The candidate will have interest for host-pathogen interactions, and a training in immunology. Some knowledge in bacteriology would also be appreciated. The candidate should be ready to work on highly pathogenic bacteria, in biosafety level 3 environments and on animal models.

Speaking either English or French is mandatory.

PROJECT 12

NEW TOOL FOR VISUALIZING AND DETECTING *klebsiella pneumoniae* INFECTIONS

Keywords: aptamer, nanobody, Klebsiella pneumoniae, in vivo imaging**Department:** Microbiology
Name of the lab: *Photonic BioImaging (UtechS)*.
Head of the lab: Spencer Shorte
Ph.D. advisor: Régis Tournebize
E-mail address: regis.tournebize@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/en/team/photonic-bioimaging-utechs-pbi/
***Doctoral school affiliation and University*:** ED Bio Sorbonne Paris Cité – Université Paris Diderot

PRESENTATION OF THE LABORATORY AND ITS RESEARCH TOPICS:

The Imagopole is a research technology platform (core facility) providing expertise and support in optical imaging methods for researchers at the Institut Pasteur. Our activities include service rendering, training, technology-driven research and technology development. They are highly multi-disciplined, and collaborative, with the mission goal focused on the use of quantitative imaging and analysis to understand the processes of cell/tissue-biology, and their usurpation by infection and disease. The R&D is founded upon the need to develop optical imaging methods and tools that bring new understanding of host-pathogen interactions and in situ high-content imaging techniques and their application to infection, cell biology, cellular microbiology, and microbiology.

DESCRIPTION OF THE PROJECT:

Infectious diseases have a major impact on public health worldwide as we observe the emergence or re-emergence of diseases combined with the appearance of bacterial strains resistant to multiple antibiotics. An early and accurate diagnosis of infectious agents followed by prompt and relevant therapy are major prerequisites for a favorable disease outcome, and more and more infections require precise identification of the pathogen before an efficient therapy is started. However, this task can be sometimes difficult to perform, as, for instance, in clinical setting where the infectious site is often difficult to access and the infectious sample could be contaminated by resident flora from adjacent zones. Altogether, these observations call for new and innovative tools and methodologies that will enable rapid, specific and sensitive identification of disease-causing pathogens that can be applied to the emerging field of non-invasive molecular imaging in biomedical and clinical research (*1*).

Nowadays, a small group of ESKAPE pathogens (for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella. pneumoniae* and *Escherichia coli*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, and Enterobacter species) represent a major public health challenge, both in developed and developing countries (*2*). These pathogens are responsible for numerous pathologies in the community, and in hospital, including septicaemia, urinary and lung infections (*3*).

This project proposes to target the *E. coli* and *K. pneumoniae* species and to **develop novel molecular imaging probes** that will allow to **directly recognise and visualise them *in vivo*.**

A recent study identified the adhesin MrKA as a potent antigen for the design of vaccines, indicating that this outer membrane protein is easily accessible. Moreover MrkA is largely conserved among Enterobacteriaceae. These observations thus identify MrkA as good candidate for selecting components targeting this protein. Such components will be able to recognise a large number of Gram negative pathogenic bacteria.
Two different types of targeting moieties will be selected during this PhD project:

- Aptamers, short oligonucleotides sequences that are capable of binding to specific targets with very high efficiency,
- Nanobodies, small single domain antibodies derived from camelids origin.

During this project the PhD candidate will work with the group of Marcel Hollenstein to select aptamers and will interact with the antibody generation team of Pierre Lafaye to select nanobodies. The project will consist of

- producing the MrkA adhesin,
- selecting aptamers and nanobodies,
- biochemical and biophysical characterization of the candidates (highest affinity in vitro, identification of binding site, stability…),
- labelling them with appropriate fluorophores,
- testing them for their capacity to recognise efficiently and selectively the bacteria *in vitro* and *in vivo* in models of infections using small animal optical imaging.

This work will develop further to adapting the aptamers and nanobodies to other modalities of in vivo imaging (PET, MRI…) via external collaboration.

This cross-disciplinary work will make use of a large set of techniques ranging from molecular biology, biochemistry, microbiology, FACS, various microscopy technologies, in vivo imaging. It will be conducted in a team with experience in physiopathology and imaging of infection diseases (*4-7*), and will benefit from the input and support of several platforms and units from the campus.

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4. L. M. S. Lery *et al.*, Comparative analysis of Klebsiella pneumoniae genomes identifies a phospholipase D family protein as a novel virulence factor. *BMC biology*. **12**, 41 (2014).

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7. R. Tournebize *et al.*, Magnetic resonance imaging of Klebsiella pneumoniae-induced pneumonia in mice. *Cell Microbiol*. **8**, 33–43 (2006).

EXPECTED PROFILE OF THE CANDIDATE

Candidates are expected to have good knowledge and experience in biochemistry, molecular biology, and possibly microbiology. They should be willing to work with animal or have previous experience in animal experimentation and handling.