Pasteur Foundation Summer Internship – Di Nunzio

Laboratory Head:

Di Nunzio, Francesca

Name of Laboratory:

Molecular Virology and Vaccinology

Title of the research project

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

Description of the research project

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

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

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

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

Tutor/supervisor

First name, Last name	Francesca Di Nunzio
Profile on http://www.researchgate.net/ (if applicable):	https://www.researchgate.net/profile/Francesca_Di_Nunzio

(Virology Lab, cont.)

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

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

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

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

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

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

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

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

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

(* equal contribution; ✉ corresponding author)

Scientific or technical background required for the research project

We are looking for highly motivated and team player individuals with a strong motivation to learn

- virology
- biochemistry
- cell biology
- molecular biology

Pasteur Foundation Summer Internship - Alzari

Laboratory Head:

Alzari, Pedro / Gubellini, Francesca

Name of Laboratory:

Structural Microbiology

Title of the research project

Protein-protein interaction in Type 7 Secretion Systems

Description of the research project

Type 7 secretion systems (T7SSs) have been discovered in *Mycobacterium tuberculosis* where they secrete key virulence factors necessary for tuberculosis infection. These secretion machineries are composed by six different proteins, four of which are inserted into the bacterial membrane. Despite its importance in the virulence of *M. tuberculosis*, the mechanism of this secretion machinery remains to date completely unknown. Investigation of the structure and the function of T7SSs are instrumental to understand their molecular mechanisms of transport, and their implication in pathogenicity.

This project aims to investigate the interactions between the membrane proteins forming the 'core' of the *M. tuberculosis* T7SSs. During this internship the student will use bacterial double hybrid technique to identify binary proteins interactions. This will be followed by co-purification of the target proteins and first structural analysis of the identified complexes by electron microscopy.

The obtained results will indicate for the first time which membrane components interact and may form stable sub-complexes of T7SS. Therefore they will be critical to the structural analysis allowing unveiling how the T7SSs are organized.

The project will be carried out in the group of Pedro ALZARI, leader in structural biology of mycobacterial proteins. This is part of a broader project lead by Dr. Francesca GUBELLINI, who will train the internship student together with a second-year PhD student (M. TASSINARI). This project is carried out in collaborations with other groups among which the one of Rémi FRONZES (Institut Européen de Chimie et Biologie, Bordeaux) and the group lead by Roland BROSCH (Institut Pasteur, Paris).

Tutor/supervisor

First name, Last name	**Name of Laboratory:** Structural Microbiology ***Laboratory Head:*** *Pedro ALZARI* ***Internship Tutor:*** *Francesca GUBELLINI*
Profile on http://www.researchgate.net/ (if applicable):	https://www.researchgate.net/profile/Francesca_Gubellini

(Structural Microbiology Lab, cont.)

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

1. Wojtowicz H, Prochnicka-Chalufour A, de Amorim GC, Roudenko O, Simenel C, Malki I, Pehau-Arnaudet G, Gubellini F, Koutsioubas A, Pérez J, Delepelaire P, Delepierre M, Fronzes R, Izadi-Pruneyre N. Structural basis of the signalling through a bacterial membrane receptor HasR deciphered by an integrative approach. (2016) Biochem J. 473(14):2239-48.
2. Goyal P, Krasteva PV, Van Gerven N, Gubellini F, Van den Broeck I, Troupiotis-Tsaılaki A, Jonckheere W, Pehau-Arnaudet G, Pinkner JS, Chapman MR, Hultgren SJ, Howorka S, Fronzes R & Remaut H. Structural and mechanistic insights into the bacterial amyloid secretion channel CsgG (2014) **Nature**, 516(7530):250-3.
3. Low HH*, Gubellini F*, Rivera-Calzada A, Braun N, Connery S, Dujeancourt A, Lu F, Redzej A, Fronzes R, Orlova EV, and Waksman G. Structure of a Type IV Secretion System. (2014) **Nature**, 508(7497). Highlighted in: Nature review microbiology 2014, 12 (5). (* equal contribution).

Scientific or technical background required for the research project

Experience in basic techniques of molecular biology (PCR, bacterial transformation and DNA purification) and/or biochemistry (protein-protein interactions, purification) is preferable but not mandatory. Good organizational and communication skills will be required.

Pasteur Foundation Summer Internship - Manina

Laboratory Head:

Manina, Giulia

Name of Laboratory:

Developmental and Stem Cell Biology

Title of the research project

Image analysis within a single-cell-based screening for drugs that fine-tune mycobacterial heterogeneity

Description of the research project

Our Junior Group of *Microbial Individuality and Infection* is striving to elucidate the non-genetic phenotypic heterogeneity of mycobacteria in its broadest sense, in the context of persistent tuberculosis infection, which remains a major global health challenge (http://www.who.int/tb/publications/global_report/en/). Our working hypothesis is that the higher is cell-to-cell heterogeneity the higher is the likelihood to survive in the presence of different stress conditions, including antibiotics. This summer project will be inserted within an ongoing endeavor, which is carried out by a research engineer in the lab. Notably, we are currently developing a microfluidic platform that will enable us to independently control multiple microwells, aiming to culture a mycobacterial fluorescent reporter strain, and expose individual microcolonies to a library of small molecule compounds. The global goal of the project is to identify one or a few compounds that can decrease the level of cell-to-cell heterogeneity, in other words reduce the coefficient of variation of bacterial fluorescence, aiming to homogenize the whole population and make it more uniformly susceptible to an approved anti-tubercular drug. Paired with the research engineer, the summer student will have the opportunity to carry out time-lapse microfluidic microscopy, as well as he will be actively involved in analyzing the image stacks produced. Multi-parametric single-cell analysis will be crucial in understanding which molecule will be worth to follow up and test in combination with the anti-tubercular drug. In sum, this project ultimately aims to identify a novel drug combination that may become in the future a fast-acting regimen, which is urgently needed.

Tutor/supervisor

First name, Last name	**Giulia MANINA**
Profile on http://www.researchgate.net/	https://www.researchgate.net/profile/Giulia_Manina https://research.pasteur.fr/en/team/microbial-individuality-and-infection/

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

Publications (†Corresponding author, *Equal contribution) – 510 citations, *h*-index: 9

5. Dhar N, McKinney J, **Manina G**† (**2016**) Phenotypic Heterogeneity in *Mycobacterium tuberculosis*. ***Microbiol Spectrum*** *ASM* 4(6):TBTB2-0021-2016. (doi:10.1128/microbiolspec.TBTB2-0021-2016).
4. Dhar N, **Manina G** (**2015**) Single-cell analysis of mycobacteria using microfluidics and time-lapse microscopy. ***Methods Mol Biol*** 1285:241–56.
3. **Manina G**†, Dhar N, McKinney JD (**2015**). Stress and host immunity amplify *Mycobacterium tuberculosis* phenotypic heterogeneity and induce nongrowing metabolically active forms. ***Cell Host & Microbe*** 17(1):32–46.
2. **Manina G**†, McKinney JD (**2013**) A single-cell perspective on Non-Growing but Metabolically Active (NGMA) bacteria. ***Curr Top Microbiol*** 374:135–61.
1. Makarov V*, **Manina G***, Mikusova K*, Möllmann U*, Ryabova O, *et al.* (**2009**) Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis. ***Science*** 324(5928): 801–4.

Patents

- Riccardi G, **Manina G**, Pasca MR. An effective new drug target for the treatment of tuberculosis. **PCT/EP2008/001088**.
- Riccardi G, **Manina G**, Pasca MR. Nitroreductase NfnB from *Mycobacterium smegmatis*. **PCT/EP2008/009231**.

Scientific or technical background required for the research project

Computational biology and image analysis.

Pasteur Foundation Summer Internship – Sauvonnet

Laboratory Head:

Sauvonnet, Nathalie, and Ferrari, Mariana

Name of Laboratory:

Microbial Individuality and Infection

Project Proposal

Title of the research project

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

Description of the research project

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

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

Tutor/supervisor

First name, Last name	NATHALIE SAUVONNET FERRARI Mariana
Profile on http://www.researchgate.net/ (if applicable):	https://www.researchgate.net/profile/Nathalie_Sauvonnet https://research.pasteur.fr/fr/team/molecular-microbial-pathogenesis/

(Microbial Individuality Lab, cont.)

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

1. Basquin C, Trichet M, Vihinen H, Malardé V, Lagache T, Ripoll L, Jokitalo E, Olivo-Marin JC, Gautreau A, Sauvonnet N. 2015. Membrane protrusion powers clathrin-independent endocytosis of interleukin-2 receptor. EMBO J. 2015 Aug 13;34(16):2147-61
2. Mounier J, Boncompain G, Senerovic L, Lagache T, Chrétien F, Perez F, Kolbe M, Olivo-Marin JC, Sansonetti P, Sauvonnet, N. 2012. Cholesterol relocation induced by the *Shigella* virulence factor IpaB inhibits host cell secretion by disrupting the Golgi complex and recycling network. Cell Host and Microbe, Sep 13;12(3):381-9.
3. Ferrari ML & P Sansonetti. 2016. Encyclopedia of Cell Biology. Cellular Invasion by Bacterial Pathogens. 2016, Pages 784–793

Scientific or technical background required for the research project

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

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

Pasteur Foundation Summer Internship – Wain-Hobson

Laboratory Head:

Wain-Hobson, Simon / Suspène, Rodolphe

Name of Laboratory:

Molecular Microbial Pathogenesis

Project Proposal

Title of the research project

On the road to cancer via cell stress

Description of the research project

Cancer is a disease of the genome, aka a pile of thousands to millions of mutations, indels and rearrangements. Over the last five years it has emerged that the human genome encodes two DNA mutator enzymes, one of which is interferon induced. It is now accepted that they are as important as UV light and the mutagens in tobacco smoke. This is hugely important for cancer most commonly emerges on a background of chronic inflammation – just think of hepatitis B and C viruses or *Helicobacter pylori*. In short, the danger is often within our cells. Our lab is working on these two mutator enzymes, APOBEC3A and APOBEC3B, the former being by far the more important clinically. Its physiological role is to degrade intracytoplasmic DNA that arises from stressed mitochondria. The project will define how genotoxic, oxidative, endoplasmic reticulum stress and infections impact the mitochondrial network and resulting in rupture and the presence of intracytoplasmic mitochondrial DNA which is then read as a danger signal. Via interferon induction of APOBEC3A the genome should be wounded and this in a non-tumoral setting. Iterate this process of cell stress coupled to genome mutation and inevitably a Darwinian process of selection will ensue. The emergence of an anti-social cell that outgrows its neighbours is axiomatic. Techniques will involve high throughput DNA sequencing, cell and molecular biology as well as imaging.

Tutor/supervisor

First name, Last name	Rodolphe Suspène
Profile on http://www.researchgate.net/ (if applicable):	

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

Caval et al., Nucl Acids Res 2015 43: 9340
Caval et al., Nat Commun 2014 5: 5129
Caval et al., Mol Biol Evol 2014 31: 330
Mussil et al., PLoS One 2013 8: e73641
Suspène et al., PLoS One 2013 8: e63461
Aynaud et al., J Biol Chem 2012 287: 39182
Suspène et al., Proc Natl Acad Sci USA 20 11 108: 4858

Scientific or technical background required for the research project

No specific background is required. The student should be interested in the biomedical sciences, enjoy for hard work and thinking apart from being motivated.