**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Interplay between polarity proteins, junction complexes and regulators of Rho-GTPases in the control of apical contraction during hematopoietic stem cell emergence from the aorta floor in the zebrafish embryo

**Keywords**: Hematopoiesis, stem cells, zebra fish, live cell imaging, Cell and Developmental Biology

**Department:** Developmental and Stem Cell Biology

**Name of the lab:** Macrophages and Development of Immunity

**Head of the lab:** Philippe Herbomel

**PhD advisor:** Anne Schmidt

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***Doctoral school affiliation and University*:** ED Complexité du Vivant, Université Pierre et Marie Curie

Presentation of the laboratory and its research topics:

Our laboratory, headed by Philippe Herbomel, is studying developmental hematopoiesis and host-virus interactions, two essential aspects of immunity. We are using the zebrafish, which is a model organism offering numerous and unique advantages in comparison to other vertebrates such as the transparency of embryos and larvae that allow live-imaging at relatively high resolution in a non invasive manner. The zebrafish also provides easiness and diversity regarding transgenesis and many other genetic approaches.

The host-virus interactions axis (team headed by Jean-Pierre Levraud), studies anti-viral innate response orchestrated by interferons (IFNs) that are secreted upon viral detection. The team has characterized zebrafish IFNs (very similar to ours) and identified their receptors and downstream stimulated genes such as the TRIM (TRIpartite Motif) genes. Models of experimental viral infection responses of the zebrafish are also being developed such as the ones triggered by Infectious Hematopoietic Necrosis Virus (a salmonid rhabdovirus) and the human mosquito-borne virus (Chikungunya virus).

The developmental hematopoiesis axis (team headed by Philippe Herbomel), studies two fundamental aspects of definitive hematopoiesis that will lead to the genesis of all blood cells in the adult: the emergence of hematopoietic stem and progenitor cells (HSCs/HSPCs) from the dorsal aorta floor, according to the so-called endothelial-to-hematopoietic transition (EHT) and the establishment of the caudal hematopoietic niche (the equivalent of the foetal liver in mammals). The team has pioneered the description, using live imaging, of the successive events starting from the EHT process *per se* and ending with the seeding of HSPCs in the thymus and the kidney marrow (the final hematopoietic niche in the adult which is the equivalent of the mammalian bone marrow).

Finally, the two teams are also studying the function and behaviour of macrophages and neutrophils in vivo, during pathogen invasion and in the context of the embryonic and larval development.

The laboratory is composed of 5 permanent scientists, 2 post-docs, 4 PhD students, 2 engineers, 5 technical and 1 administrative staff members. We also host, on regular basis, several trainees from different Universities worldwide.

Description of the project:

During vertebrate embryonic development, hematopoietic stem cells (HSCs) emerge from vascular components and, in particular, the aorta wall. The process is relatively well conserved through vertebrate species, appears to be very unique in its topology and cellular dynamics, and is characterized by unusual morphological changes such as the bending of both apical and basal cellular membranes in the same direction. This process was identified in our laboratory using the zebrafish embryo and named the endothelial-to-hematopoietic transition (EHT, ref1).

Currently, not much is known about the molecular and intracellular signalling events controlling EHT and we wish to unravel those aspects in order to comprehend a very fundamental and original aspect of Stem Cell Biology. In addition, our work should strongly contribute to the knowledge required for producing HSCs in vitro for the purpose of regenerative medicine, which still remains a challenge.

In the recent past years, our laboratory has developed high-resolution optical imaging approaches to improve visualizing EHT in time and space. We also set up analytical and molecular tools to unravel essential molecular events controlling the morphological changes leading to EHT and to study inter-cellular junction complexes engaged with and between surrounding endothelial cells whose organization and dynamics must compromise with the maintenance of vascular integrity. Recently, we have obtained high-resolution 2-D maps of the vascular landscape surrounding EHT cells with a detailed cartography of inter-cellular junction densities and associated sub-cortical actin cytoskeleton. We have characterized the dynamics of a circumferential acto-myosin belt surrounding the apical side of EHT cells whose contraction is essential for the emergence (in preparation, ref2).

1. This PhD project will be aimed at deciphering, in the context of EHT, the molecular interplay between intercellular junctions, cell polarity complexes and the regulators of acto-myosin activity. This axis of our hematopoiesis research topic will be privileged because two of the hallmarks of EHT (and in comparison to other cell extrusion mechanisms, see ref3) are the reinforcement of junction complexes until the very end of the process and gain in polarity (unpublished results). Both should cooperate for the recruitment of highly specific regulators of Rho GTPases to ensure the spatio-temporal control of acto-myosin activity that is key to the proper evolution of apical contraction of EHT cells. Depending on the status of ongoing work, the project will articulate more or less between (1) the identification of Rho GTPases regulatory exchange factors (Rho-GEFs/GAPs) that are effector proteins of the Crb or of the Par polarity complexes and that are involved in the acto-myosin belt contraction during EHT (several candidates are currently under scrutiny including p114Rho-GEF and its regulators, targets of atypical PKC (a Par complex member)), (2) the identification of junction proteins interacting with polarity proteins and expressed in EHT cells (ex: JAMs, nectins). We will address the specific expression of the mRNAs and proteins of interest in the zebrafish aortic hemogenic endothelium using (a) in situ hybridization, (b) a challenging technical approach using the CRISPR/Cas9 technology in order to insert an HA-tag (single tag or tandem repeat) in the ORF of variants to investigate their expression in the hemogenic endothelium and determine their subcellular localization (ref4), (c) immunofluorescence and confocal microscopy. Other methodologies for functional analysis will be used such as RNA interference and mutant protein expression in zebrafish embryos after transgenesis, reverse transcription, PCR, cDNA cloning and, if necessary, zebrafish transgenic lines will be established. Finally, the project may also require the use of optical approaches for cell restricted spatio-temporal control of interfering protein expression and cell tracing to track more specifically newly born HSCs and progenitors (ex: infrared laser-mediated gene induction, ref5).

References:

1. *Kissa K., & Herbomel P. (2010). Nature, 464(7285), 112–115.*
2. *Lancino M., Majello S., Schmidt A. & Herbomel P. acto-myosin contraction controls hematopoietic stem cell emergence in the zebra fish embryo. In preparation.*
3. *Gudipaty S. A., & Rosenblatt J. (2016). Seminars in Cell & Developmental Biology.*
4. *Hruscha A. et al. (2013). Development, 140(24), 4982–4987.*
5. *Kamei et al., (2009). Nature Methods, 6(1), 79-81.*

Expected profile of the candidate (optional):

Ideally, the candidate should have a Development, Cell and Molecular Biology background and good knowledge in Cell Imaging and Biochemistry.

Contact:

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