**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:** 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

**PhD advisor:** Sandrine Etienne-Manneville

**Email address:** setienne@pasteur.fr

**Web site address of the lab:** www.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,

1. 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.
2. to characterize the role of evolutionary conserved polarity proteins, including Cdc42, Scrib, APC (Adenomatous Poliposis Coli) and Dlg1 (Discs Large), in the signalling 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.
3. to determine how polarity signalling 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 thenanoscale 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 tumour 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:

1. *Dupin, I., and Etienne‐Manneville, S. (2011). Nuclear positioning: mechanisms and functions. Int J Biochem Cell Biol 43, 1698‐1707.*
2. *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.*
3. *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.*
4. *Leduc, C., and Etienne‐Manneville, S. (2015). Intermediate filaments in cell migration and invasion: the unusual suspects. Curr Opin Cell Biol 32, 102‐112.*
5. *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 (optional):

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

Contact:

Sandrine Etienne-Manneville : setienne@pasteur.fr