Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : Function of the Actin-Spectrin Cytoskeleton in Morphogenesis: From Molecular Structure to Cellular Dynamics

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Unité de Recherche :
Intitulé : **Laboratoire de Biologie du Développement**
Code : **CNRS UMR7622**
Equipe de Recherche :
Intitulé : **Cortical actomyosin dynamics in development and morphogenesis (CADMO)**
Thématique de recherche : Cell dynamics and morphogenesis from molecule to tissue
Responsable d'équipe :
NOM : **ROBIN** Prénom : **François**
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **Complexité du Vivant (ED515)**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :
3 doctorants encadrés : Serena Prigent (2017, 100%), Anne Van Gorp (2017, 50%), Karen Lorena Chauca Espinoza, (2020, 100%)

CO-DIRECTION *(obligatoire)*

Co-Directeur de Thèse (titulaire d'une HDR) :
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Unité de Recherche :
Intitulé : **Biomolécules: Analyse, Interactions Moléculaires et Cellulaires**
Code: UMR 7203
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Biomolécules: Analyse, Interactions Moléculaires et Cellulaires**
Thématique de recherche : Development of biophysical and chemical biology tools to understand the functions of biomolecules and their interactions in various biological processes
Responsable d'équipe :
NOM : **Burlina** Prénom : **Fabienne**
Ecole Doctorale de rattachement : **ED388 – Chimie Physique, Chimie Analytique**

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :
3 doctorants encadrés : Hela Ben Aissa (2017 - 100%), Fanny Broch (2018 - 100%), Louise-Marie Rakotoarison (2019 - 100%)

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Précisez ici les éventuels co-encadrants (non HDR)

Co-encadrant :
NOM : **LLENSE** Prénom : **Flora**
Titre : **Maitre de conférence** HDR ☐
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Unité de Recherche :
Intitulé : Laboratoire de Biologie du Développement
Code : UMR7622
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Forces mécaniques et morphogenèse des tissus**
Thématique de recherche : Morphogenèse de la cellule au tissu
Responsable d'équipe :
NOM : **LABOUESSE** Prénom : **Michel**
Ecole Doctorale de rattachement : ED515
Ou si ED non SU :

Résumé (2 000 caractères maximum) : Morphogenesis results from the balance between coordinated cell behavior and homeostasis of cell group to achieved complex 3D form of tissues, organs and organisms. How local cell shape changes drive cell division or tissue deformation such as elongation during embryonic development is a fundamental question. During embryogenesis, actomyosin is a major determinant of the mechanical properties of the cell, and drives morphogenesis at the cell and tissue level. This active material forms a complex cross-linked network beneath the cell surface – the cell cortex – that turns over rapidly and is weakly organized. Spectrins in particular play an important role in the architecture and dynamics of the cortex.

First identified as the major component of the erythrocyte membrane cytoskeleton, the spectrin meshwork is formed by heterodimeric head-to-head units of α and β-spectrin, assembled side-to-side in an antiparallel manner. The resulting tetramers crosslinking F-actin is named spectrin-actin network. In physiological conditions, spectrins play a pivotal functional role in the dynamics of the actin cytoskeleton, both during cell division and morphogenesis. In *C. elegans* only three spectrins are present, each encoded by a single gene: β-G spectrin (*unc-70*), β-H(heavy) spectrin (*sma-1*) and α-spectrin (*spc-1*). Even though little is known concerning their structural organization during development, *sma-1* and *spc-1* have been identified as critical regulators of cell mechanics. How these large structural molecular units (300nm) are organized *in vivo*, and how this structural organization affects the dynamics of the actin cytoskeleton, however, still remains unclear. To shed light on this question and explore the functional importance of spectrins in embryonic development and morphogenesis, we will use an interdisciplinary, multiscale approach, combining genetics, state-of-the-art microscopy techniques and chemical biology, to implement new tools for improved microscopy approaches.

Joindre en annexe un descriptif du PRD avec références au format pdf
(« NOM_2_IPV_2021 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :

à envoyer simultanément par e-mail à l'ED de rattachement et au programme :
interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 février minuit.

Function of the Actin-Spectrin Cytoskeleton in Morphogenesis: From Molecular Structure to Cellular Dynamics

Morphogenesis results from the balance between coordinated cell behavior and homeostasis of cell group to achieved complex 3D form of tissues, organs and organisms. How local cell shape changes drive cell division or tissue deformation such as elongation during embryonic development is a fundamental question. During embryogenesis, actomyosin is a major determinant of the mechanical properties of the cell, and drives morphogenesis at the cell and tissue level. This active material forms a complex cross-linked network beneath the cell surface – the cell cortex – that turns over rapidly and is weakly organized. Spectrins in particular play an important role in the architecture and dynamics of the cortex.

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First, we aim to visualize the dimers of spectrin *in vivo*. To this is end, we will take a two-tier approach. On the one hand, we will use live microscopy to visualize already available fluorescent fusions α -spectrin with mKate (SPC-1::mKate) and β_H-spectrin with GFP (SMA-1::GFP). We will visualize living embryos expressing SPC-1::mKate or/and SMA-1::GFP by time-lapse video imaging using a spinning-disc confocal microscopy from early division to 2 fold stage. From this we could confirm the expression level and the localization over a long time scale. In order to resolve how the spectrins may associate structurally, the two ends separated from 300nm of the α (SPC-1::mKate) and β (Sma-1::GFP) spectrin dimer can be observed under Total Inflection Reflected Fluorescence (TIRF) microscopy. To resolve these structures spatially in live embryos, we will also use Structured Illumination Microscopy TIRF (SIM-TIRF), a super-resolution microscopy which goes beyond the light diffraction limit and whose spatial resolution is two folds higher than TIRF, in order to infer the orientation of both spectrins at the cell cortex. Using particle tracking, we will infer from these data the orientation of the spectrin dimers, and further characterize the stoichiometry of the complexes and their dynamics, focusing two specific developmental stages, the early embryo and the elongating embryo[1]. **These approaches however display some severe limitations in their scope, as their spatial resolution is limited (~70nm on SIM-TIRF) and they do not provide information on the orientation of the probes.**

Second, we will therefore turn to chemical biology to expand the reach of our existing microscopy techniques. To better visualize the spectrin-actin network, we will develop new imaging techniques leveraging the properties a newly developed Fluorogen Activating and absorption-Shifting Tag (FAST). FAST is a chemogenetic fluorescent marker that fluoresces upon the reversible binding of fluorogenic chromophores[2].

(a) The visualization of sparse structures with a limited number of individual molecules, such as actin (~360 monomers/1μm) or spectrins (detailed stoichiometry unknown, but likely <6), along with the use of large biological objects (antibodies/DNA) are two core reasons limiting the precise super-resolution observation of a number biological structures. We will combine FAST with PALM/STORM-like Single-Molecule Localization Microscopy (SMLM) super-resolution technique. of the spectrin network to visualize network architecture with higher resolution. Indeed, similar to the PAINT technique, the reversible and dynamic exchange between the FAST and the fluorogenic chromophore provides an essentially unlimited source of localization events, with the simple requirement of generating a fluorescent probe. Furthermore, given its small size, the offset between the sample and the probe is limited to a very small distance. FAST was shown to be suitable for various super-resolution microscopy techniques such as SMLM[3], SRRF[4] and STED[5]. To fully optimize its use for SMLM, we will optimize the probe using a concerted strategy involving in silico modeling and directed evolution to tune its exchange rate and increase its photostability. Using this newly improved FAST, we will then implement FAST-SMLM in our system, initially targeting the visualization of the spectrin architecture in fixed specimens with nm accuracy, or the actin network, to observe functional defects in network architecture in specific set of mutants for either elongation or cell division.

(b) While the visualization of network structure using for example SIM-TIRF has increased largely, information regarding network orientation remains scarce. Recently, techniques using polarization microscopy have re-emerged as a powerful approach to further explore network organization. In fluorescence polarization microscopy, a fluorophore is rigidly coupled to its bait, preventing rotation of the fluorophore with respect to the bait. The orientation of the fluorescence dipole is then used as a readout of the orientation of the bait: under illumination by a linearly polarized laser, the emission of the fluorophore is then tightly coupled to the orientation of the laser. We previously demonstrated that we could precisely explore the architecture of actin networks using polarization microscopy in live embryos using engineered fusions of LifeAct with GFP (Robin group in collaboration with Manos Mavrakis and Sophie Brasselet at the Institut Fresnel, Marseille). Our results were somewhat limited by the photostability of the probe, and could therefore not be expanded to super-resolution microscopy. Here, we propose to develop new fluorescence probes combining FAST with polarization microscopy to explore the spatial orientation of spectrin-actin networks in live embryos.

Third, we explore the role of spectrin-actin networks in embryonic development during two well-defined embryological contexts: the first cell division, and embryo elongation. To this end, we will aim to identify the main functional partners of such macromolecular assemblies and how they do differ in morphogenetic processes. Then, we will analyze the effect of other actin crosslinkers known to functionally interact with spectrins (*plst-1*, *fhod- 1*, *gsln-1*) on the organization of the spectrin cytoskeleton at these two key developmental stages. At 1-cell stage, s*pc-1* RNAi-knockdown increased pulsed contractility in the cortex[6]. Furthermore, while the loss-of-

function of the plastin/fimbrin PLST-1 delays the cytokinesis[7], the combined knock-down of *sma-1* and *plst-1* completely blocks cell division (Ana Carvahlo, unpublished data). During the elongation, *sma-1(-)* slows the morphogenetic process while *spc-1(-)* stops the development at 2-fold stage[8]. We will characterize under the differential interference contrast (DIC) microscope the defect in the cytokinesis or in elongation length of: *sma-1(ru18)* and *spc-1(ra409)* null mutants, *plst-1*(RNAi) and *spc-1+plst1* (RNAi) embryo. To shed light on the SMA-1 and SPC-1 genetic partners, we will then perform gene-candidate enhancer or suppressor RNAi screen on *spc-1 and or sma-1* mutant background (null starting allele) with a collection of cytoskeleton genes, notably actin-binding proteins and signaling proteins, including those already known to interact with its SH3 domain[8]. This will help to identified new genetics partners which belong to the same regulatory networks and act in a related pathway.

In conclusion, this project will combine genetics, existing microscopy tools and the development of new microscopy techniques using biological chemistry technologies, to better explore the architectural organization and function of the spectrin-actin network during embryonic morphogenesis. We expect that the candidate will grow at the interface between cell and developmental biology and chemical biology, and eventually contribute to the emergence of a new generation of scientists that can both develop tools and use them in the lab.

Supervision. The PhD candidate will be co-supervised by Dr. François Robin and Flora Llense (Laboratoire de Biologie du Développement), and Prof. Arnaud Gautier (Laboratoire des Biomolécules). François Robin is a cell biologist interested in understanding how mechanical forces drive cell change shape during embryonic development and morphogenesis. His research focuses on the biological processes that control the mechanical properties of cells, focusing on the actomyosin cortex. CRCN at Inserm (2016) and recruited as group leader on an open call at IBPS (2016), he an ATIP-Avenir laureate (2015). Flora Llense is a cell and developmental biologist. Recruited in 2016 as *Maître de Conférence*, she has a broad expertise in *C. elegans* biology, genetics and fluorescence microscopy. Arnaud Gautier is a chemical biologist developing chemogenetic tools for biological imaging combining the power of modern chemistry with advanced directed evolution approaches. He developed the FAST technology for *in vivo* protein labeling. He is laureate of the CNRS bronze Medal (2017), and is a junior member of IUF (since 2018).

1. F. B. Robin *et al.*, *Nat Meth.* **11**, 677–682 (2014).
2. M.-A. Plamont *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **113**, 497–502 (2016).
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4. M. Venkatachalapathy *et al.*, *Nanoscale.* **11**, 3626–3632 (2019).
5. H. Benaissa *et al.*, *bioRxiv.* **12**, 2021.01.29.428635 (2021).
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