Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : Mechanical Polarity of the Cell Cortex

Directeur de Thèse porteur du projet (titulaire d'une HDR) :
NOM : **du Roure** Prénom : **Olivia**
Titre : Directrice de Recherche
e-mail : olivia.duroure@espci.fr
Adresse professionnelle : PMMH
Sorbonne Université
Barre Cassan, Bat A premier étage,
7 Quai Saint Bernard 75005 Paris

Unité de Recherche :
Intitulé : Physique et Mécanique des Milieux Hétérogènes, CNRS, ESPCI, Université de Paris, Sorbonne Université
Code : UMR7636
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **BioPhysique cellulaire**
Thématique de recherche : Biophysique et mécanique du cytosquelette
Responsable d'équipe : Olivia du Roure et Julien Heuvingh
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED564 PIF**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :
Lucas Prévost : 2018, 50%
Zhibo Li : 2019, 50%
Magdalena Kopec : 2019 , 50%
Joseph Vermeil : 2020, 50%

CO-DIRECTION *(obligatoire)*

Co-Directeur de Thèse (titulaire d'une HDR) :
NOM : **Piel** Prénom : **Matthieu**
Titre : Directeur de recherche HDR ☒
e-mail : Matthieu.piel@curie.fr

Unité de Recherche :
Intitulé : Cell Biology and Cancer, CNRS, Intitut Curie, PSL
Code: UMR144
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Systems Biology of Cell Division and Cell Polarity**
Thématique de recherche : Biologie cellulaire
Responsable d'équipe : Mathieu Piel
Ecole Doctorale de rattachement :
Ou si ED non SU : **ED 577 SDSV**

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :
Alice Williart (ED SDSV) 2019 (50%, co-encadrement N. Manel).

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

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Co-encadrant :
NOM : **Heuvingh** Prénom : **Julien**
Titre : Maître de Conférences HDR non
e-mail : julien.heuvingh@espci.fr
Unité de Recherche :
Intitulé : PMMH
Code : UMR 7636
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **BioPhysique cellulaire**
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Responsable d'équipe : Olivia du Roure et Julien Heuvingh
Ecole Doctorale de rattachement : ED PIF

Résumé (2 000 caractères maximum) :
Les cellules mammifères peuvent adopter une grande variété de formes lors de leur migration ou pendant la morphogenèse des tissus. Ces formes sont souvent associées à une localisation bien définie des composants cellulaires, appelée polarité cellulaire. La capacité des cellules à contrôler leur forme et leur polarité repose en grande partie sur des réseaux dynamiques du cytosquelette et en particulier sur une fine couche de filaments d'actine associée à la membrane plasmique : le cortex d'actine. Dans ce projet de thèse, nous proposons de comprendre comment, en plus des composants moléculaires, les propriétés mécaniques du cortex cellulaire peuvent également être polarisées, et comment cette polarité contribue à la morphogenèse d'une cellule unique et d'un seul tissu.
Nous avons développé une nouvelle technique, basée sur l'attraction dipolaire entre micro-billes superparamagnétiques, qui permet de sonder les propriétés mécaniques et dynamiques du cortex d'actine. Grâce à cette technique et à l'imagerie de fluorescence, nous voulons étudier l'établissement de la polarité cellulaire, à la fois sur cellules uniques et sur un modèle de tissu. Sur une cellule unique, nous voulons étudier l'établissement de la polarité apico-basale lorsque les cellules adhèrent sur un substrat ainsi que la polarité avant/arrière en utilisant des cellules en migration et des techniques d'opto-génétiques qui établissent artificiellement une telle polarité. En poursuivant la même démarche expérimentale sur de doublets de cellules puis des epithelium, nous chercherons à comprendre comment le cortex se modifie et se polarise lors de l'établissement des jonctions cellules-cellules, ainsi que l'établissement d'une polarité entre le cortex de cellules au bord et au cœur du tissu qui sont centrales dans les phénomènes de migration collective.

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 :

Avis favorable

Ecole Doctorale
Physique en Ile de France
ED PIF - ED n° 564
Pr Maria Chamarro
Directeur-adjoint SU

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.

Mechanical Polarity of the Cell Cortex

Biological context

Mammalian cells can adopt a large variety of shapes when migrating or during tissue morphogenesis. These shapes are often associated with a well-defined localization of cellular components, called cell polarity. The capacity of cells to control their shape and their polarity relies in part on dynamic cytoskeletal networks and in particular on a thin layer of actin filaments associated to the plasma membrane, the actin cortex. Both the molecular composition of the actin cortex and its mechanical properties have gained a lot of attention *(1-3)*, and the importance of this structure in morphogenetic phenomena is well established. **In this PhD project, we propose to understand how, in addition to molecular components, mechanical properties of the cell cortex can also be polarised, and how this polarity contributes to single cell and tissue morphogenesis.**

Although studies have already reported local mechanical measures of the cell surface that suggest the existence of mechanical polarity, there is little knowledge on its underlying physical and molecular mechanisms or on its prevalence in polarizing functions in cells and tissues. Which aspects of cell mechanics (membrane or cortex tension, contractile forces, etc…) are involved in cell polarity is not yet understood. We hypothesize the cell cortex is instrumental in defining a mechanical polarity of the cell and that this cortical mechanical polarity is essential for the polarization of cellular functions. For example, it was proposed that cortical flows synergize with biochemical networks to define the polarity of the C. Elegans embryo (*4*). In the same system, a local weakening of the cell cortex was proposed to define the polarity axis of the embryo and initiate the polarization process (*5*). More generally, a local thinning of the cortex can lead to the local formation of membrane detachment, or blebs, contributing to cell migration and cell division (*6*). **The goal of the PhD project is to characterize the polarity of the cell cortex and understand how it contributes to the shape and behaviour of both single cells and epithelial monolayers.**

Experimental Tools

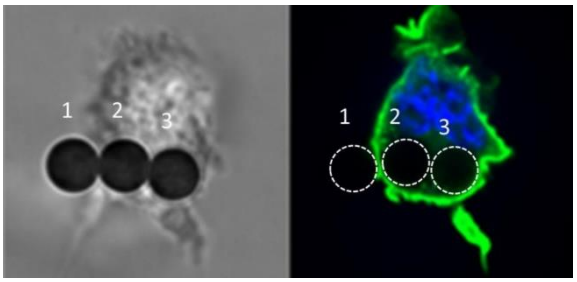
The collaborations between the two groups (PMMH and Curie/IPGG) recently resulted in the development of a method to measure the thickness of the cell cortex pinched between two magnetic beads with a controlled force (*7*). This ***magnetic pincher*** also enables the fine characterization of the rheology of the cell cortex. Using this method, we observed transient phases during which the cortex can become locally very thick or very thin. This instability depends on the activity of Myosin II, and is likely to be modulated by **local** regulation of motor activity. The project will also be based on microfabrication techniques, currently used in the IPGG team, such as micro-patterning, microchannels and cell-confiner. A great attention will be devoted to quantitative fluorescent imaging (confocal and spinning disk) of the cortex and its components in the same time as biophysical measurements with the cortex pincher. We also plan during the course of the phD to integrate opto-genetics tools via a collaboration with Mathieu Coppey's Team in Pysico-Chimie Curie Lab (Institut Curie, CNRS, Sorbonne Univ.)

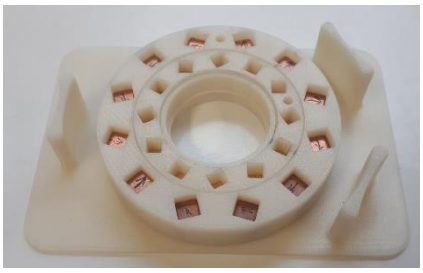
Understanding how polarity cues establish a cortical mechanical polarity in single cells

We will introduce a first level of polarity by adhesion on the extra-cellular matrix, corresponding to the apico-basal polarity in epithelial cells. A second level, corresponding to front/back in migratory cells, or antero-posterior axis in polarized epithelial, is under the control of small GTPases and their regulatory factors (8) and will be studied through confinement and optogenetic tools. We thus propose to **experimentally control, at the single cell level these two axes of polarity, and perform local measures of the cortex mechanics, to characterize its mechanical polarity**.

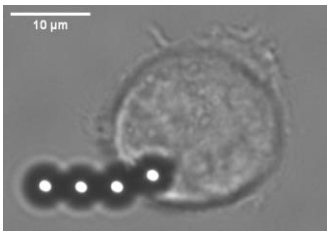
To study apico-basal polarization, we will start from the case of suspended cells, **and introduce a controlled adhesion using micropatterns of various sizes**, generating a bottom and a top cortex. The cortex properties (thickness, fluctuations, and rheology measured through the magnetic pincher, density and mesoscopic structure through fluorescent imaging) will be compared to the unpolarized floating case. Given the time resolution of our techniques, we should be able to follow the evolution of these properties during the maturation of adhesion and establishment of cortex polarization.

The concept of the magnetic pincher is based on the uptake of a large magnetic bead (several micron diameter) into a living cell. This uptake can be realized either by macropinocytosis or phagocytosis and have been tested in our groups for different cell type such as Dentritic Cells of the immune system, the unicellular organism Dictostelium and the 3T3 epithelial cell line. When an external magnetic field is imposed, beads that have not been ingested are attracted by the ingested beads through dipolar magnetic force. The self-organisation of these beads into couple or chains simplifies greatly the experiment. When the magnetic field is kept constant at a low value, the cortex thickness can be measured with an accuracy of a few tens nm. We can also measure the amplitude of the temporal fluctuations in its thickness, with a nm accuracy. This "activity" is dependent on actin polymerisation but moreover on the instabilities due to the cortex contractions via myosin motors. By varying the external magnetic field, we vary the pinching force between the colloids (up to 1.5 nanoNewton), which allows the measurement of the deformation of the cortex as a function of the applied force. We can thus obtain the elasticity and rheology of the cortex itself, in contrast to existing methods that deform the cortex from the exterior of the cell and gather contributions from the cortex but also from internal structures. In contrast to other methods, the pincher can measure the mechanics of both floating and adherent cells. Electromagnets are used in this experiments, which requires a dedicated setting. We however fabricated easy-to-use arrays of permanent magnets arranged into a ring that allows the experiment to be ported to other setups and that will be used for confocal microscopy experiments. The array shown here is made of two nested ring, that one can rotate to provide a field between <0.1 mT and 70 mT. This portable setup will guarantee that the experiments can take place in both labs as well at our collaborators' lab.

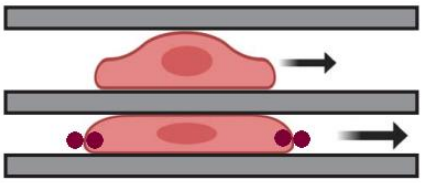




An example of the magnetic pincher used on patterned cells is given in the adjacent picture. Patterns of adhesion of relatively small size are required to avoid overspreading of the cell where the height of the cell would be smaller than the size of the beads. The bottom cortex will only be monitored through fluorescent imaging.



The front/back polarization, will be first studied using confinement: height confinement of cells by two non-adhering substrates leads to their polarization and a fast migratory behaviour *(9)*. We will take advantage of this to measure the cortex properties at different location on the polarized cortex. Given the self-organisation nature of the pincher, situations where both the leading and trailing part of the actin cortex can be we probed at the same time are likely to occur.

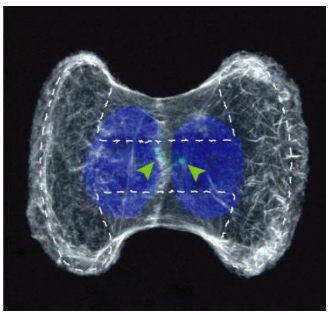


We will then perform local activation of small GTPases controlling cell polarity (Rac1, RhoA and Cdc42) using optogenetic tools. We will follow the cortex local properties during the activation and monitor them as a function of the distance to the activated region. This will characterize the spatiotemporal mechanical response of the cortex to an induced front-back polarization event. This mechanical characterization will then be performed in the context of drug treatments and molecular perturbations to assess the specific contribution of the main known components of the cell cortex. This will allow us to identify the key molecular ingredients of the cortical mechanical polarity.

This study will provide a spatio-temporal characterization of cell cortex mechanics in live adhesive cells, in response to patterns of activation of front/back polarity cues. **It will constitute the first in depth description of the mechanical polarity of the cell cortex.**

Understanding how cortex mechanics relays polarity cues to orchestrate tissue behavior

The remodeling of epithelia during tissue development, regeneration and renewal involves continuous rearrangement of intercellular contacts. In this process, contacts mechanics are instrumental, and often exhibit spatial patterning and planar polarity or anisotropy. Using FRET tension sensors in intercellular adhesion proteins of MDCK epithelia, it was shown that the pull exerted by the cortex on adhesion complexes exhibits spatial patterning in the form of a transcellular gradient in epithelia polarized by collective migration (*10*). **This suggests that a spatial patterning of cortex mechanics at cell-cell contacts directs collective behavior.**



We will first assess **how cell-cell contact formation remodels the cell cortex and shapes its mechanical polarity**. This study will be performed using MDCK cells, which are a model in vitro system for epithelial monolayers, through a collaboration with Nicolas Borghi in Institut Jacques Monod. We will start from a two-cell system plated on micropatterned surfaces (such as the adjacent picture from cit *11*). We will monitor cortex thickness and rheology by magnetic pincher at the intercellular contact with one bead in each cell, and the contact-free periphery. We will compare these proximal and distal effects to that of optogenetic-induced polarization, since RhoGTPases are involved in contact formation. In both cases, we will parallel these experiments with a measurement of actin crosslinkers recruitment. We will use Ca2+ switch (addition or removal from the medium) to perturb cell adhesion and vinculin mutants to affect actin bundling and branching at cell-cell junctions.

The long term goal of the project, once the patterned single cell and the cell doublets will be fully caracterised, is to move to a fully assembled monolayer. We will impose an additional polarity axis using either wounding-induced collective migration of the epithelium, or optogenetic activation of contractility in a subset of cells in the monolayer. We will examine the impact on cortex mechanics of contact distance and relative orientation to the epithelium migrating edge, in normal and vinculin mutants. We will ask whether cortex mechanics differs between contacts parallel and perpendicular to the epithelium edge, reflecting the difference in molecular composition (*12*) and tensional state. These experiments will **characterize the mechanical polarity of the cell cortex in polarized epithelia and reveal the reach over which cells influence each other's mechanical polarity, thus contributing to the global tissue behavior and the emergence of collective migration.**

The PhD student will be fully part of both teams and will conduct experiments in both labs thanks to the new portable set-up we developed. He/she will thus fully benefit from the experience and culture of biophysics in PMMH and cell biology in Curie/IPGG. The experimental program detailed here is ambitious, but each part (single cell polarization during adhesion, front/back polarisation, cell doublets, cell epithelia) is expected to provide results which can be independently valorized and will contribute, in various biological contexts, to the global understanding of cell polarity, a fundamental cellular property.

References

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