Mechanical regulation of vesicular protein transport from the Golgi complex to the Cell surface

To reach the cell surface, secreted proteins are transported along intracellular routes from the endoplasmic reticulum through the Golgi complex. Cargos exit the Golgi complex in transport carriers that use microtubules to be efficiently addressed to the plasma membrane before exocytosis. The Golgi complex plays a central role in secretion by processing and sorting cargos. Using a synchronized secretion assay in 2D, we have recently highlighted that exocytosis does not occur randomly at the cell surface but at hotspots juxtaposed with focal adhesions (FAs) which are major cell-matrix adhesions and can convert mechanical forces into actin contraction (1) (Fig.1). Whether mechanic-dependent functions of FA are involved in non random secretion remains unknown. However, several questions remain unanswered. Indeed, our published work and unpublished preliminary data indicate a mechanical influence from the cell environment on secretion hotspots. In addition, **the existence of exocytosis hotspots in 3D remains unknown.** Understanding the mechanical and molecular players involved in the spatial regulation of protein secretion in cells in 2D, in 3D and in polarized models is at the core of this PhD project. Finally, recent studies highlighted that the endomembrane system is sensing and responding to mechanical cues (2). Hence the microenvironment and its mechanics could regulate protein secretion via yet unknown feedback loops.

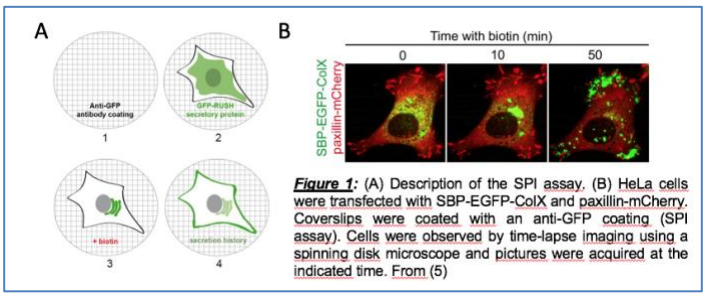

Figure 1: (A) Description of the SPI assay. (B) HeLa cells were transfected with SBP-EGFP-ColX and paxillin-mCherry. Coverslips were coated with an anti-GFP coating (SPI assay). Cells were observed by time-lapse imaging using a spinning disk microscope and pictures were acquired at the indicated time. From (5)

The general aim of this interdisciplinary PhD project is to understand the crosstalk between the mechanics of the cell microenvironment and the regulation of protein secretion from the Golgi to the plasma membrane.

The PhD project is divided in 3 specific and complementary parts:

1/ How mechanical properties of the substrate influence protein secretion to the cell surface at secretion hotspots in 2D? Is there a feedback loop between the Golgi and adhesion complexes?; 2/ How intracellular trafficking routes adapt to cell polarity and mechanical forces?; 3/ Can we understand, recapitulate and predict the crosstalk between trafficking and traction force in 2D and 3D based on a physical model?

General experimental system: To assess protein secretion in 2D, we will analyze synchronized protein transport using the RUSH (Retention using Selective hooks) (3) assay coupled to TIRF microscopy and Spinning Disk Microscopy. We will expose cells to multiple types of mechanical forces including stretching, compression and substrate rigidity. Dynamic analysis of living samples will be developed in tight collaboration with the Imaging facility of the Institut Curie.

Aim #1: How mechanical properties of the substrate influence protein secretion to the cell surface at secretion hotspots in 2D? In 2D, we have shown that protein secretion occurs at hotspots associated to FAs (1). However, we observed that not all FAs sustain exocytosis (1). One question we aim to answer now is: what determines that a FA becomes a secretory hotspot and that another does not? FAs are dynamic by nature; they constantly grow and disassemble. In this aim, we propose to test the influence of two parameters on the capacity for FAs to sustain exocytosis: i) the age of FAs and ii) their response to mechanical forces. We plan to monitor protein secretion using the RUSH assay allowing synchronization of secretory cargos (3). We will then modulate applied force by stretching and see how it influences secretion at FAs.

In addition, FAs act as mechanotransducers through the cytoskeleton, as FA proteins connect integrins to the actin cytoskeleton. We will assess the coupling between integrin, actin and

membrane arrival at secretion hotspots. To do this, we will follow and quantify in parallel actin dynamics and coupled traction forces, arrival of secretory vesicles and Integrin Beta1 at FAs using the RUSH system.

Experimental approaches: The turnover rate of FAs will be measured by TIRF microscopy using fluorescently tagged paxillin for several hours before inducing cargo transport. The use of asymmetrical fibronectin micropatterns and stretching will allow to control and modulate adhesion forces (Fig. 2A) We have already designed a photomask to generate asymmetrical patterns and validated that FAs of different shape and size are formed. Using this set-up, we will then monitor protein secretion using the RUSH assay. To modulate the forces, we will either change substrate stiffness or perform a uniaxial stretch using dedicated cell stretchers on cells plated on fibronectin coated micropatterned silicon thin film. This technic allows to apply a controlled deformation on cells where the cell adhesion geometry is given via the micropattern (Fig.2B). In addition, forces exerted by the different FAs will be measured using traction force microscopy that we already implemented with success in our team (Fig.3).

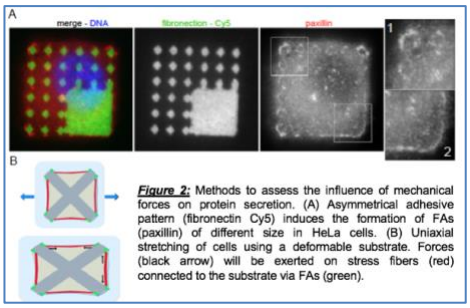

Figure 2: Methods to assess the influence of mechanical forces on protein secretion. (A) Asymmetrical adhesive pattern (fibronectin Cy5) induces the formation of FAs (paxillin) of different size in HeLa cells. (B) Uniaxial stretching of cells using a deformable substrate. Forces (black arrow) will be exerted on stress fibers (red) connected to the substrate via FAs (green).

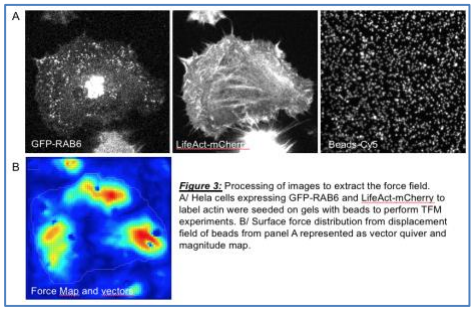

Figure 3: Processing of images to extract the force field. A/ Hela cells expressing GFP-RAB6 and LifeAct-mCherry to label actin were seeded on gels with beads to perform TFM experiments. B/ Surface force distribution from displacement field of beads from panel A represented as vector quiver and magnitude map.

Aim#2: How intracellular trafficking routes adapt to cell polarity and mechanical forces?

We will integrate our previous results to a 3D polarized model (4) and we will modulate the mechanical forces by compression, matrix with various stiffness, osmotic shocks and self-rolling substrates (5) (corresponding to monolayer of epithelial MDCK cells plated on self-rolling substrates which allow a transient increase of cell volume, see III) and follow their impact on Golgi to plasma membrane trafficking.

How intracellular trafficking routes adapt to cell polarity? In 2D, the Golgi-associated RAB6 is a reporter of post-Golgi secretion (1) and we showed that RAB6-positive vesicles are moving along MTs and are highly dynamic (6, 7). However, in a 3D environment, live cell imaging of post-Golgi trafficking (RAB6 marker) has never been performed. Combining specific imaging techniques (long-distance objectives, high speed multicolour time-lapse spinning-disk confocal microscopy) and cells grown in 3D (MDCK 3D cysts and epithelial monolayers) will enable us to draw a precise map of post-Golgi vesicles trajectories (RAB6 and RUSH cargos) in parallel to MTs dynamics and polarity.

How intracellular trafficking routes adapt to mechanical forces? In parallel to the use of regular 3D-cysts that we will submit to different external constrains, we will take advantage of a new set-up recently developed (5) which leads to an acute and transient change in mechanical forces that will allow us to understand how intracellular trafficking routes adapt to mechanical forces. By comparing epithelial monolayer on flat and curved conditions with the use of the self-rolling substrates (see III for details), we will decipher how post-Golgi trafficking (RAB6 and RUSH cargoes) behaves and adapts upon change in mechanical forces.

This aim represents a challenge in setting up the experimental and imaging conditions but will bring high gain in knowledge in the field of polarized transport.

Experimental approaches: To follow the dynamics of RAB6-post Golgi vesicles as well as RUSH cargos in a 3D environment, we will use high speed multicolour time-lapse spinning-disk confocal microscopy of thick samples coupled to long-distance objectives. This will enable us to describe the dynamics of post-Golgi vesicles (GFP-RAB6) combined to Microtubules dynamics (EB3-GFP) as well as RUSH cargos together in 3D cysts and in epithelial MDCK monolayers. To modulate external

forces in 3D cysts, we will exert external forces using matrix with different stiffness. In monolayer of epithelial MDCK cells plated on self-rolling substrates, we will increase transiently cell volume in a time-scale of 10 min which is compatible with imaging of RAB6-positive vesicles as well as pulse of trafficking with the RUSH system.

Aim#3: Modeling the crosstalk between trafficking and traction force in adherent and motile cells? In 2D adherent cells, we will develop theoretical models to identify feedback loops between traction forces and the intracellular recycling of adhesion components. Cellular traction forces are based on active stress generation by the acto-myosin cytoskeleton and transient coupling to the substrate via the stochastic binding and unbinding of adhesion complexes. Physical models of this process have so far overlooked the need to recycle components of adhesion complexes. We will introduce recycling via microtubule-based transport into traction force models. We will predict the correlation between the local actin dynamics, the local flux of recycling vesicle at the adhesion site, and the traction force, and directly compare our prediction with the experimental observations (which has been evidenced in 8). In 3D epithelial monolayer and cyst, the model of force generation developed in 2D will be adapted to the symmetric situation of tension generation at cell-cell junctions. We will establish the requirement on trafficking and junction remodeling needed to prevent symmetry breaking between neighboring cells and the loss of intercellular tension. Based on our quantitative predictions, we will propose experimental perturbations of membrane trafficking that could lead to the loss of monolayer integrity that will be then tested.

Experimental approaches: We will adapt the so-called “molecular clutch” model of force generation by actin retrograde flow to the actin dynamics observed near FA, and include the dynamics of adhesion molecules via a diffusion-advection equation with recycling. Feedback between local stress and recycling will be introduced *via* a phenomenological coupling between the microtubule and actin networks. The model will yield coupled dynamical equations between trafficking and force generation which will be studied using the classical tools of dynamical system to identify fixed points (steady-states) and study their stability under various perturbation accessible experimentally to compare with the observations of aims #1 and #2.

Thanks to this ambitious PhD proposal, we will uncover how vesicular trafficking, MTs, FAs and secretion are organized and can be predicted in a nearly physiological context and respond to mechanical forces.

Scientific environnement

Aims #1 and #2 of the PhD project imply the analysis of the dynamics of RAB6 vesicles and of synchronized protein transport. These two aspects are being analyzed for several years in the team. In addition, the PhD candidate will employ other imaging techniques (such as TIRF microscopy, super-resolution, etc...) which are available at the imaging facility of the Institut Curie with whom we work in tight collaboration. The majority of experimental procedures are already implemented in the laboratories and secured (traction force microscopy, fibronectin patterns…). The PhD candidate will also have the opportunity to set-up new tools such as stretching of cells and cell volume changes using monolayers of MDCK cells. These developments will be done in collaboration with the teams who developed these new tools. Aims #2 and #3 will be supervised by Pierre Sens, (DR1 CNRS, Institut Curie, Paris), who is a theoretical physicist with a strong expertise in modeling biological soft active matter and the active mechanics of cells, with a focus on cytoskeleton-membrane interaction, cell adhesion and cell motility.

The two partners have complementary set of skills in cell biology and theoretical physics and have been collaborating for several years, which will insure effective synergy between them.

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