Unit : Physical chemistry « Curie » - UMR 168 CNRS/Institut Curie
Group : Biomimetic Systems Of Cell Motility
Group leader: C. SYKES

The ultimate goal of our work is to understand how cells move, with implications for the treatment of metastatic cancer. We develop biomimetic systems that reproduce cell movement under controlled conditions. Using this approach, we can study the physical and biochemical mechanisms governing cell movement. We have successfully mimicked actin-based propulsion, and we are now going a step further and including myosin molecular motors in the system as well as deformable membranes. In parallel, we are working on other biopolymers that induce movement by self-assembly such as the MSP (Major Sperm Protein) cytoskeleton. MSP polymerization is the basis of sperm cell movement in the nematode Caenorhabditis elegans, as these sperm cells contain no actin and no myosin.

Biomimetic systems of cell movements

The cell cytoskeleton is composed of actin filaments, microtubules and intermediate filaments. Actin filaments are dynamic, undergoing constant polymerization, depolymerization and rearrangement, and thereby power the deformation and movement of the cell. Over the last ten years, much progress has been made toward understanding the biochemical components of localized actin polymerization and organization. However how actin polymerization generates the force to propel a cell remains a much debated topic, as forces produced by polymerization of single filaments are on the order of pico Newtons, whereas the forces involved in cell movement are estimated to be on the nano Newton scale. To study how nanometer sized actin filaments are organized into micron-sized structures in the cell, we will use a dual microscopic/macroscopic approach while varying biochemical parameters. The goal is to understand how force is generated on both the microscopic and macroscopic level, the biochemical dependence of force generation, and how individual filament dynamics are integrated to produce forces and movements in the cell. The ultimate objective is the production of an artificial cell where actin filaments (or other biopolymer structures) exert forces on the membrane and result in movement.

To this end, we design biomimetic systems where actin polymerization is reproduced in a controlled fashion on surfaces by coating with actin polymerization activators. These surfaces include hard beads, soft beads and inner or outer leaflets of lipid bilayers in liposomes. The objects are then incubated in cell extracts or in pure protein mixes and the actin structures that grow from the surfaces mimic cellular filamentous actin. Actin structures thus formed can be observed by light microscopy (Fig 1) or electron microscopy. This set-up lends itself to quantitative measurements and physical characterization as parameters can be varied, such as the size and deformability of the beads, the composition of the protein mix and the nature and density of the bead/liposome coating. Thus we can test the physical and biochemical properties of actin polymerization in order to better understand how polymerization produces motility and membrane deformations.

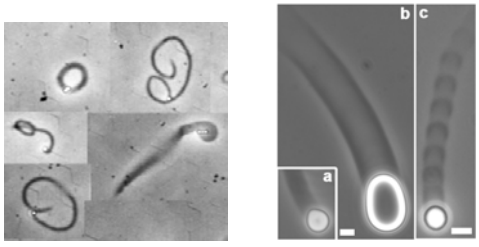

Fig 1: Left: 0.5 µm diameter beads coated asymmetrically with a protein that activates actin polymerization in the cell (the VCA domain of WASP), incubated in HeLa cell extracts. The beads are propulsed by the comet of polymerized actin that forms. The actin comets appear dark. Phase contrast microscopy. C. Sykes unpublished results. Right: Soft beads deformed (a,b) or jumping (c) due to the stresses exerted by the actin comet. The beads are coated with VCA, and beads (b) and (c) recruit also the VASP protein. Bars 2 µm. Trichet et al., 2007.

The role of Ena/VASP proteins in motility and adhesion (J. Plastino and co-workers)

Trichet, L., Sykes, C., Plastino, J. (2008) Relaxing the actin cytoskeleton for adhesion and movement with Ena/VASP J. Cell Biol. 181: 19-25. PhD project of Philippe Noguera)

We use the bead system to study the interplay of two molecules that are key for cell motility and adhesion: the Arp2/3 complex and Ena/VASP protein. These proteins are partially colocalized in the cell, but play antagonistic roles in that Ena/VASP proteins reduce the frequency of Arp2/3 complex-based actin branch structures. Recently we showed that the overall effect of Ena/VASP proteins on the actin network is to detach filaments from activating proteins, perhaps due to Ena/VASP anti-branching activity. We propose that these two activities (anti-branching and detaching) are key for the maturation of cell-cell junctions and essential for the cell shape changes that occur during embryogenesis (Fig 2). We intend to study the mechanistic basis of these activities by measuring how different phosphorylation states and mutants of Ena/VASP affect bead speeds and comet structure. In parallel, we will perform complementary studies in the embryo of the nematode Caenorhabditis elegans in order to evaluate the effect of different forms of Ena/VASP on cell-cell adhesion during embryogenesis.

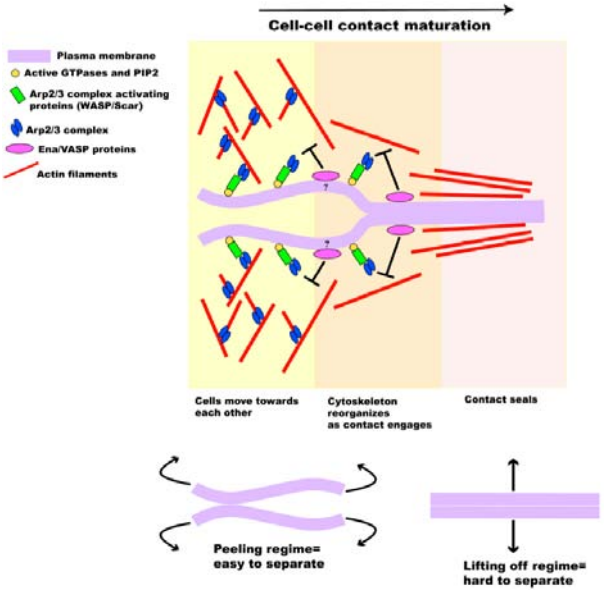

Fig 2: Schematic representation of the possible role of Ena/VASP proteins in the reorganization of the cytoskeleton for cell-cell adhesion. Ena/VASP detaches filaments from the membrane and the network collapses into a belt beneath the membrane, reinforcing adhesion. Trichet et al., 2008.

Reconstituting an actin cortex in a liposome (C. Sykes and co-workers)

Pontani L.-L.., J. Van der Gucht, G. Salbreux, J. Heuvingh, C. Sykes. 2009. in press Biophysical Journal.

In order to more closely mimic the living cell, we are currently developing new systems composed of liposomes filled with the minimum motility components. Actin polymerization activators are attached to the inner leaflet of the lipid bilayer of artificial liposomes, and the interior of the lipsosome is filled with the ingrediants necessary for actin polymerization. The liposomes are produced by a reverse emulsion technique that uses very little material and avoids the use of electric fields as for classic electroformation of liposomes. We have succeeded in forming a dynamic actin cortex (Fig 3). The thickness of the cortex grows with time and is dependent on the size of the liposome. This system provides a simplified and controlled model of cellular actin polymerization at the membrane.

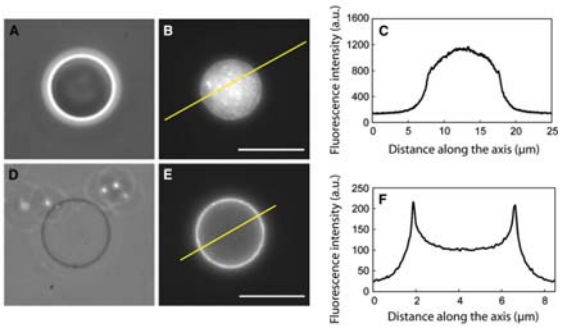

Fig 3: Phase contrast (A, D) and fluorescence (B, E) images of liposomes. The curves (C, F) correspond to the linescans shown in yellow in B and E, respectively. In A and B, fluorescent monomeric actin is encapsulated in the liposome with sucrose (to enhance contrast). The entire interior of the liposome appears fluorescent. Insertion of pores in the membrane allows flows of salt and sucrose across the membrane (D) and triggers actin polymerization in a cortex at the membrane (E). Bar: (A et B) 10 m, (C, D) 5 m. Pontani et al., 2009.

Mechanical measurements of the biomimetic actin cortex: fluctuations (C. Sykes and coworkers, post-doc T. Betz)

In order to characterize the properties of our artificial cortexes, and compare them to cells, we have constructed a novel set-up to measure membrane fluctuations of liposomes.

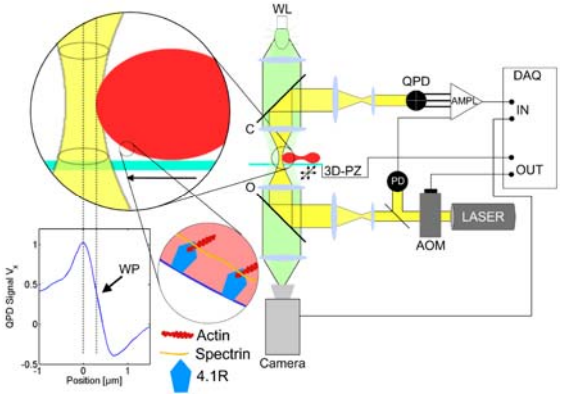

Fig 4: Set-up for measuring membrane fluctuations of liposomes. T. Betz, unpublished results

An optical tweezers is focused on the membrane, and fluctuations are measured using a 4-quadrant diode (Fig 4). We have tested the set-up on red blood cells, and we were able to show that the rigidity varies depending on the presence of ATP and is affected by drugs that act on the actin cytoskeleton.

Mechanical measurements of the biomimetic actin cortex: micropipets and pulling tubes (C. Sykes and coworkers, post-doc C. Campillo)

In collaboration with the the group of Patricia Bassereau, we are characterizing the mechanical properties of cortex-containing liposomes by a micropipet aspiration technique (Fig 5). The length of bulge pulled into the micropipette can be related to the mechanical properties of the membrane/cortex, and by comparing liposomes with and without cortex, we can define the properties of the biomimetic actin cortex. Preliminary results indicate that the bulge in the presence of the cortex is much larger than without cortex.

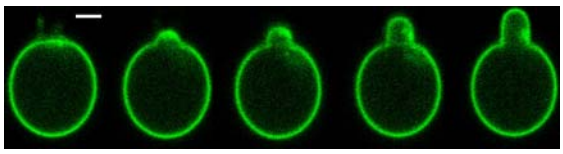

Fig 5: A liposome with a cortex is being aspirated into a micropipet. Actin appears in green. Bar 3 µm. C. Campillo, unpublished results.

Formin-based actin polymerization on membranes (C. Sykes and coworkers, post-doc M. Safouane)

We have developed a novel system for observing membrane deformations induced by formin-based actin polymerization. We observe that membrane deformations are larger when actin is organized into bundles. We are now quantifying the force necessary to deform the membrane from the outside, and subsequent work will involve incorporating formins inside liposomes to characterize membrane deformations from internal forming-based actin polymerization.

The cytoskeleton of C. elegans sperm cells (J. Plastino and coworkers)

In parallel with actin, we are studying another biopolymer system capable of producing forces and movement, the MSP (Major Sperm Protein) cytoskeleton of nematode sperm cells (Fig 6). For sperm cell movement, MSP fills the role of actin, while the analog of myosin remains unknown. This system provides an interesting model for biopolymer-based movement.

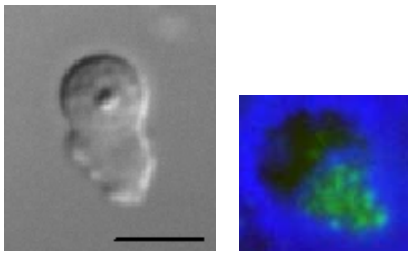

Fig 6: A C. elegans sperm cell is crawling on the substrate by extending a lamellipodia Bar 2 m. J. Plastino, unpublished results. On the right, a fixed sperm cell, immunolabeled vor MSP (in green). The membrane extensions which drive cell movement are filled with MSP. E. Batchelder, unpublished results.

Our ultimate goal is to understand how MSP polymerization produces movement, to characterize the physical and biochemical mechanisms, and to compare the MSP system to the actin system. We are currently identifying the activators of MSP polymerization. By immunolabeling, we have observed that a phosphotyrosine protein is present at the end of membrane extensions containing MSP, and we are working on identifying this protein. In addition, we have produced a transgenic worm expressing fluorescent MSP in collaboration with the laboratory of Erik Jorgensen Utah University, USA). We are using this worm to visualize MSP dynamics during movement.