Jamecna D, Polidori DJ, Mesmin B, Dezi M, Lévy D, Bigay J, Antonny B (2019 Mar 22)

An intrinsically disordered region in OSBP acts as an entropic barrier to control protein dynamics and orientation at membrane contact sites


**Résumé**

Lipid transfer proteins (LTPs) acting at membrane contact sites (MCS) between the ER and other organelles contain domains involved in heterotypic (e.g. ER to Golgi) membrane tethering as well as domains involved in lipid transfer. Here, we show that a long ≈ 90 aa intrinsically unfolded sequence at the N-terminus of oxysterol binding protein (OSBP) controls OSBP orientation and dynamics at MCS. This Gly-Pro-Ala-rich sequence, whose hydrodynamic radius is twice as that of folded domains, prevents the two PH domains of the OSBP dimer from homotypically tethering two Golgi-like membranes and considerably facilitates OSBP in-plane diffusion and recycling at MCS. Although quite distant in sequence, the N-terminus of OSBP-related protein-4 (ORP4) has similar effects. We propose that N-terminal sequences of low complexity in ORPs form an entropic barrier that restrains protein orientation, limits protein density and facilitates protein mobility in the narrow and crowded MCS environment.

Bertin Aurélie, Lomakin Alexis (2019 Feb 15)

**Meeting report – Building the Cell 2018**

*journal of cell science*: 132 : [DOI: 10.1242/jcs.229765]

**Résumé**

Cell biologists from all around the world gathered in Paris on the 26 to 28 September 2018 to participate in the 3rd international meeting ‘Building the Cell’. It was organized by Hélène Barelli, Arnaud Echard, Thierry Galli, Florence Niedergang, Manuel Théry and Marie Hélène Verlhac on behalf of the French Society for Cell Biology (SBCF) at the Institut Pasteur. Around 230 participants joined the meeting for stimulating talks, discussions, poster sessions, and a gala dinner on the Seine that included a music performance by the rock group ‘Membrane Band’. The unifying theme of the meeting was the development of creative multidisciplinary approaches to understand cellular life at different scales in a dynamic and quantitative manner. Here, we summarize the results presented at the meeting and the emerging ideas from the different sessions.

The 3rd international meeting ‘Building the Cell’ ([Fig. 1](#); [Fig. 2](#)) was divided into ten different sessions that covered a variety of topics including intracellular trafficking, cell division, cytoskeletal dynamics and cell mechanics, cancer and stem cell biology, embryonic development and tissue morphogenesis, neurobiology, and aggregates and phase transitions. A broad spectrum of modern approaches and experimental systems ranging from synthetic biology and stem cell technologies to 3D organoids and animal models was presented. The meeting highlighted some of the latest and novel findings in cell biology,
often coupled to major methodological developments in quantitative microscopy and computational modelling, as well as cell and tissue micro-engineering.

**Membrane reshaping by micrometric curvature sensitive septin filaments**
*Nature communications*: DOI: 10.1038/s41467-019-08344-5

**Résumé**

Septins are cytoskeletal filaments that assemble at the inner face of the plasma membrane. They are localized at constriction sites and impact membrane remodeling. We report in vitro tools to examine how yeast septins behave on curved and deformable membranes. Septins reshape the membranes of Giant Unilamellar Vesicles with the formation of periodic spikes, while flattening smaller vesicles. We show that membrane deformations are associated to preferential arrangement of Septin filaments on specific curvatures. When binding to bilayers supported on custom-designed periodic wavy patterns displaying positive and negative micrometric radii of curvatures, septin filaments remain straight and perpendicular to the curvature of the convex parts, while bending negatively to follow concave geometries. Based on these results, we propose a theoretical model that describes the deformations and micrometric curvature sensitivity observed in vitro. The model captures the reorganizations of septin filaments throughout cytokinesis in vivo, providing mechanistic insights into cell division.

**Actin dynamics drive cell-like membrane deformation.**
*Nature Physics*: Accepted

**Résumé**

Cell membrane deformations are crucial for proper cell function. Specialized protein assemblies initiate inward or outward membrane deformations that turn into, for example, endocytic intermediates or filopodia. Actin assembly and dynamics are involved in this process, although their detailed role remains controversial. We show here that a dynamic, branched actin network is sufficient to initiate both inward and outward membrane deformation. With actin polymerization triggered at the membrane of liposomes, we produce inward filopodia-like structures at low tension, while outward endocytosis-like structures are robustly generated regardless of tension. Our results shed light on the mechanism of endocytosis, both in mammalian cells, where actin polymerization forces are required when membrane tension is increased, and in yeast, where those forces are necessary to overcome the opposing turgor pressure. By combining experimental observations with physical modeling, we propose a mechanism for actin-driven endocytosis controlled by membrane tension and the architecture of the actin network.
The depolymerase activity of MCAK shows graded response to Aurora B kinase phosphorylation through allosteric regulation.
journal of cell science: DOI: 10.1242/jcs.228353

Résumé
Kinesin-13 motors regulate precise microtubule dynamics and limit microtubule length throughout metazoans by depolymerizing microtubule ends. Recently, MCAK has been proposed to undergo large conformational changes during its catalytic cycle, as it switches from solution to bound state. Here, we reveal that MCAK has a compact conformation in solution using crosslinking and electron microscopy. When MCAK is bound to the microtubule ends, it adopts an extended conformation with the N terminus and neck region of MCAK interacting with the microtubule. Interestingly, the region of MCAK that interacts with the microtubule is the region phosphorylated by Aurora B and contains an EB-binding motif. The level of phosphorylation of the N terminus results in a graded microtubule depolymerase activity. Here we show the N terminus of MCAK forms a platform to integrate Aurora B kinase downstream signals and in response fine-tunes its depolymerase activity during mitosis. We propose that this allosteric control mechanism allows decoupling of the N terminus from the motor domain of MCAK to allow MCAK depolymerase activity at kinetochores.

Feng-Ching Tsai*, Aurelie Bertin*, Hugo Bousquet, John Manzi, Yosuke Senju, Meng-Chen Tsai, Laura Picas, Stephanie Miserey-Lenkei, Pekka Lappalainen, Emmanuel Lemichez, Evelyne Coudrier*, Patricia Bassereau* (2018 Sep 30)
Ezrin enrichment on curved membranes requires a specific conformation or interaction with a curvature-sensitive partner.
elife: 7: e37262: DOI: 10.7554/eLife.37262

Résumé
One challenge in cell biology is to decipher the biophysical mechanisms governing protein enrichment on curved membranes and the resulting membrane deformation. The ERM protein ezrin is abundant and associated with cellular membranes that are flat, positively or negatively curved. Using in vitro and cell biology approaches, we assess mechanisms of ezrin’s enrichment on curved membranes. We evidence that wild-type ezrin (ezrinWT) and its phosphomimetic mutant T567D (ezrinTD) do not deform membranes but self-assemble anti-parallelly, zipping adjacent membranes. EzrinTD’s specific conformation reduces intermolecular interactions, allows binding to actin filaments, which reduces membrane tethering, and promotes ezrin binding to positively-curved membranes. While neither ezrinTD nor ezrinWT senses negative curvature alone, we demonstrate that interacting with curvature-sensing I-BAR-domain proteins facilitates ezrin enrichment in negatively-curved membrane protrusions. Overall, our work demonstrates that ezrin can tether membranes, or be targeted to curved membranes, depending on conformations and interactions with actin
and curvature-sensing binding partners.


**ProLIF – quantitative integrin protein-protein interactions and synergistic membrane effects on proteoliposomes.**
*Journal of Cell Science*: [DOI: 10.1242/jcs.214270]

**Résumé**

Integrin transmembrane receptors control a wide range of biological interactions by triggering the assembly of large multiprotein complexes at their cytoplasmic interface. Diverse methods have been used to investigate interactions between integrins and intracellular proteins, and predominantly include peptide-based pulldowns and biochemical immuno-isolations from detergent-solubilised cell lysates. However, quantitative methods to probe integrin-protein interactions in a more biologically relevant context where the integrin is embedded within a lipid bilayer have been lacking. Here, we describe ‘protein-liposome interactions by flow cytometry’ (denoted ProLIF), a technique to reconstitute recombinant integrin transmembrane domains (TMDs) and cytoplasmic tail (CT) fragments in liposomes as individual subunits or as αβ heterodimers and, via flow cytometry, allow rapid and quantitative measurement of protein interactions with these membrane-embedded integrins. Importantly, the assay can analyse binding of fluorescent proteins directly from cell lysates without further purification steps. Moreover, the effect of membrane composition, such as PI(4,5)P₂ incorporation, on protein recruitment to the integrin CTs can be analysed. ProLIF requires no specific instrumentation and can be applied to measure a broad range of membrane-dependent protein-protein interactions with the potential for high-throughput/multiplex analyses.


**Adhesion to nanofibers drives cell membrane remodeling through 1D wetting**
*Nature Communications*: [DOI: org/10.1101/393744]

**Résumé**

The shape of cellular membranes is highly regulated by a set of conserved mechanisms. These mechanisms can be manipulated by bacterial pathogens to infect cells. Human endothelial cell plasma membrane remodeling by the bacterium *Neisseria meningitidis* is thought to be essential during the blood phase of meningococcal infection, but the underlying mechanisms are unknown. Here we show that plasma membrane remodeling...
occurs independently of Factin, along meningococcal type IV pili fibers, by a novel physical mechanism we term « one dimensional » membrane wetting. We provide a theoretical model that gives the physical basis of 1D wetting and show that this mechanism occurs in model membranes interacting with model nanofibers, and in human cells interacting with model extracellular matrices. It is thus a new general principle driving the interaction of cells with their environment at the nanoscale that is diverted by meningococcus during infection.


**Septin-based readout of PI(4,5)P2 incorporation into membranes of giant unilamellar vesicles**

*Cytoskeleton*: [DOI: 10.1002/cm.21480](https://doi.org/10.1002/cm.21480)

**Résumé**

Septins constitute a novel class of cytoskeletal proteins. Budding yeast septins self-assemble into non-polar filaments bound to the inner plasma membrane through specific interactions with L-α-phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2). Biomimetic in vitro assays using Giant Unilamellar Vesicles (GUVs) are relevant tools to dissect and reveal insights in proteins-lipids interactions, membrane mechanics and curvature sensitivity. GUVs doped with PI(4,5)P2 are challenging to prepare. This report is dedicated to optimize the incorporation of PI(4,5)P2 lipids into GUVs by probing the proteins-PI(4,5)P2 GUVs interactions. We show that the interaction between budding yeast septins and PI(4,5)P2 is more specific than using usual reporters (phospholipase Cd1). Septins have thus been chosen as reporters to probe the proper incorporation of PI(4,5)P2 into giant vesicles. We have shown that electro-formation on platinum wires is the most appropriate method to achieve an optimal septin-lipid interaction resulting from an optimal PI(4,5)P2 incorporation for which, we have optimized the growth conditions. Finally, we have shown that PI(4,5)P2 GUVs have to be used within a few hours after their preparation. Indeed, over time, PI(4,5)P2 is expelled from GUV membrane and the PI(4,5)P2 concentration in the bilayer decreases.

Année de publication : 2017


**Activity of the purified plant ABC transporter NtPDR1 is stimulated by diterpenes and sesquiterpenes involved in constitutive and induced defenses.**


**Résumé**

Within the plant ABC transporter family, pleiotropic drug resistance (PDR) transporters play essential functions, such as in hormone transport or defense against biotic and abiotic...
stresses. NtPDR1 from Nicotiana tabacum has been shown to be involved in the constitutive defense against pathogens through the secretion of toxic cyclic diterpenes such as the antimicrobial substrates cembrene and sclareol from the leaf hairs (trichomes). However, direct evidence of an interaction between NtPDR1 and terpenes is lacking. Here, we stably expressed NtPDR1 in N. tabacum BY-2 suspension cells. NtPDR1 was purified as an active monomer glycosylated at a single site in the third external loop. NtPDR1 reconstitution in proteoliposomes stimulated its basal ATPase activity from 21 to 38 nmol Pi.mg⁻¹.min⁻¹, and ATPase activity was further stimulated by the NtPDR1 substrates cembrene and sclareol, providing direct evidence of an interaction between NtPDR1 and its two substrates. Interestingly, NtPDR1 was also stimulated by capsidiol, a sesquiterpene produced by N. tabacum upon pathogen attack. We also monitored the transcriptional activity from the NtPDR1 promoter in situ with a reporter gene and found that while NtPDR1 expression was limited to trichomes under normal conditions, addition of methyl jasmonate, a biotic stress hormone, induced expression in all leaf tissues. This finding indicated that NtPDR1 is involved not only in constitutive but also in induced plant defenses. In conclusion, we provide direct evidence of an interaction between the NtPDR1 transporter and its substrates and that NtPDR1 transports compounds involved in both constitutive (diterpenes) and induced (sesquiterpenes) plant defenses.


Résumé

How cellular organelles assemble is a fundamental question in biology. The centriole organelle organizes around a nine-fold symmetrical cartwheel structure typically ~100 nm high comprising a stack of rings that each accommodates nine homodimers of SAS-6 proteins. Whether nine-fold symmetrical ring-like assemblies of SAS-6 proteins harbour more peripheral cartwheel elements is unclear. Furthermore, the mechanisms governing ring stacking are not known. Here we develop a cell-free reconstitution system for core cartwheel assembly. Using cryo-electron tomography, we uncover that the Chlamydomonas reinhardtii proteins CrSAS-6 and Bld10p together drive assembly of the core cartwheel. Moreover, we discover that CrSAS-6 possesses autonomous properties that ensure self-organized ring stacking. Mathematical fitting of reconstituted cartwheel height distribution suggests a mechanism whereby preferential addition of pairs of SAS-6 rings governs cartwheel growth. In conclusion, we have developed a cell-free reconstitution system that reveals fundamental assembly principles at the root of centriole biogenesis.

Frédéric Toussaint, Baptiste Pierman, Aurélie Bertin, Daniel Lévy, Marc Boutry (2017 Mar 17) *Purification and biochemical characterization of NpABCG5/NpPDR5, a plant pleiotropic drug resistance transporter expressed in Nicotiana tabacum BY-2*
suspension cells.
The Biochemical journal : 1689-1703 : DOI : 10.1042/BCJ20170108

Résumé

Pleiotropic drug resistance (PDR) transporters belong to the ABCG subfamily of ATP-binding cassette (ABC) transporters and are involved in the transport of various molecules across plasma membranes. During evolution, PDR genes appeared independently in fungi and in plants from a duplication of a half-size ABC gene. The enzymatic properties of purified PDR transporters from yeast have been characterized. This is not the case for any plant PDR transporter, or, incidentally, for any purified plant ABC transporter. Yet, plant PDR transporters play important roles in plant physiology such as hormone signaling or resistance to pathogens or herbivores. Here, we describe the expression, purification, enzymatic characterization and 2D analysis by electron microscopy of NpABCG5/NpPDR5 from Nicotiana plumbaginifolia, which has been shown to be involved in the plant defense against herbivores. We constitutively expressed NpABCG5/NpPDR5, provided with a His-tag in a homologous system: suspension cells from Nicotiana tabacum (Bright Yellow 2 line). NpABCG5/NpPDR5 was targeted to the plasma membrane and was solubilized by dodecyl maltoside and purified by Ni-affinity chromatography. The ATP-hydrolyzing specific activity (27 nmol min(-1) mg(-1)) was stimulated seven-fold in the presence of 0.1% asolectin. Electron microscopy analysis indicated that NpABCG5/NpPDR5 is monomeric and with dimensions shorter than those of known ABC transporters. Enzymatic data (optimal pH and sensitivity to inhibitors) confirmed that plant and fungal PDR transporters have different properties. These data also show that N. tabacum suspension cells are a convenient host for the purification and biochemical characterization of ABC transporters.

Année de publication : 2016


Résumé

Mapping the conformational landscape of G protein-coupled receptors (GPCRs), and in particular how this landscape is modulated by the membrane environment, is required to gain a clear picture of how signaling proceeds. To this end, we have developed an original strategy based on solution-state nuclear magnetic resonance combined with an efficient isotope labeling scheme. This strategy was applied to a typical GPCR, the leukotriene B4 receptor BLT2, reconstituted in a lipid bilayer. Because of this, we are able to provide direct evidence that BLT2 explores a complex landscape that includes four different conformational states for the unliganded receptor. The relative distribution of the different states is modulated by ligands and the sterol content of the membrane, in parallel with the changes in the ability of the receptor to activate its cognate G protein. This demonstrates a
conformational coupling between the agonist and the membrane environment that is likely to be fundamental for GPCR signaling.

A Bertin, E Nogales (2016 Jul 31)
Preparant recombinant yeast septins et their analysis by electron microscopy.
Methods in cell biology : 21-34 : DOI : 10.1016/bs.mcb.2016.03.010

Résumé

Septins sont de façon conservées et essentielle eukaryotic cytoskeletal proteins that interact with the inner plasma membrane. They are involved in essential functions requiring cell membrane remodeling and compartmentalization, such as cell division and dendrite morphogenesis, and have been implicated in numerous diseases. Depending on the organisms and on the type of tissue, a specific set of septins genes are expressed, ranging from 2 to 13. Septins self-assemble into linear, symmetric rods that can further organize into linear filaments several microns in length. Only a subset of human septins has been described at high resolution by X-ray crystallography (Sirajuddin et al., 2007). Electron microscopy (EM) has proven to be a method of choice for analyzing the molecular organization of septins. It is possible to localize each septin subunit within the rod complex using genetic tags, such as maltose-binding protein or green fluorescent protein, to generate a visible label of a specific septin subunit in EM images that are processed using single-particle EM methodology. In this chapter we present, in detail, the methods that we have used to analyze the molecular organization of budding yeast septins (Bertin et al., 2008). These methods include purification of septin complexes, sample preparation for EM, and image processing procedures. Such methods can be generalized to analyze the organization of septins from any organism.

Aurélie Bertin, Eva Nogales (2016 Jan 15)
Characterization of Septin Ultrastructure in Budding Yeast Using Electron Tomography.

Résumé

Septins are essential for the completion of cytokinesis. In budding yeast, Saccharomyces cerevisiae, septins are located at the bud neck during mitosis and are closely connected to the inner plasma membrane. In vitro, yeast septins have been shown to self-assemble into a variety of filamentous structures, including rods, paired filaments, bundles, and rings (Bertin et al. Proc Natl Acad Sci U S A, 105(24):8274-8279, 2008; Garcia et al. J Cell Biol, 195(6):993-1004, 2011; Bertin et al. J Mol Biol, 404(4):711-731, 2010). Using electron tomography of freeze-substituted sections and cryo-electron tomography of frozen sections, we determined the three-dimensional organization of the septin cytoskeleton in dividing budding yeast with molecular resolution (Bertin et al. Mol Biol Cell, 23(3):423-432, 2012; Bertin and Nogales. Commun Integr Biol 5(5):503-505, 2012). Here, we describe the detailed procedures used for our characterization of the septin cellular ultrastructure.