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Force-control at cellular membranes

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Force-regulation at cellular membranes relies on dynamic molecular platforms that integrate intra- and extracellular signals to control cell shape and function. To correctly respond to a continuously changing environment, activity of these platforms needs to be tightly controlled in space and time. Over the last few years, curvature-dependent mechano-chemical signal translation—a receptor-independent signaling mechanism where physical forces at the plasma membrane trigger nanoscale membrane deformations that are then translated into chemical signal transduction cascades—has emerged as a new signaling principle that cells use to regulate forces at the membrane. However, until recently, technical limitations have precluded studies of this force-induced curvature-dependent signaling at the physiological scale. Here, we comment on recent advancements that allow studying curvature-dependent signaling at membranes, and discuss processes where it may be involved in. Considering its general impact on cell function, a particular focus will be put on the curvature-dependence of feedback loops that control actin-based forces at cellular membranes.

Keywords: actin, curvature, lipid, plasma membrane, signaling

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Introduction

Detection and precise control of mechanical forces is essential for proper cell function. While much is known about the role of individual proteins in this process (e.g. mechanotransduction via ion channels, integrins, extracellular matrix proteins, and cell adhesion molecules; reviewed in[1,2]), the contribution of cellular membranes has largely remained elusive. The plasma membrane is continuously deformed in- and outward in response to a wide range of intracellular [3,4] and intercellular [5-7] forces. In response to such nanoscale membrane deformations, cytosolic proteins and membrane lipids are enriched in a curvature-dependent manner.[8-11] Since some of the recruited molecules contain signaling properties,[12-14] this recruitment leads to the formation of local, transient signaling hubs (**Fig. 1**). This process, where physical forces applied to cellular membranes create deformations that are translated into classical chemical signal-transduction pathways, is called curvature-dependent mechano-chemical signal translation. The functional properties of such transient, curvature-dependent signaling hubs critically depend on the protein composition at these sites. As each protein has its own curvature selectivity,[15] the protein composition of signaling hubs is defined by the membrane curvature and by what subset of curvature-sensitive proteins is expressed in the specific cell.

While proteins that are recruited in a curvature-dependent manner are capable of affecting a variety of signaling processes, we will focus here on proteins that create local feedback mechanisms to control direction, amplitude and duration of force generation at bent membranes. Specifically, we aim to discuss the cause and consequences of such transient force-regulating feedback-loops, with a particular emphasis on proteins that control actin dynamics.

Bending the plasma membrane

Over the last decades the number of proteins capable of deforming the plasma membrane inward and outward has continued increasing.[3,11,16] As we start to understand the mechanisms how forces required to bend the membrane are generated, an overarching theme is emerging:

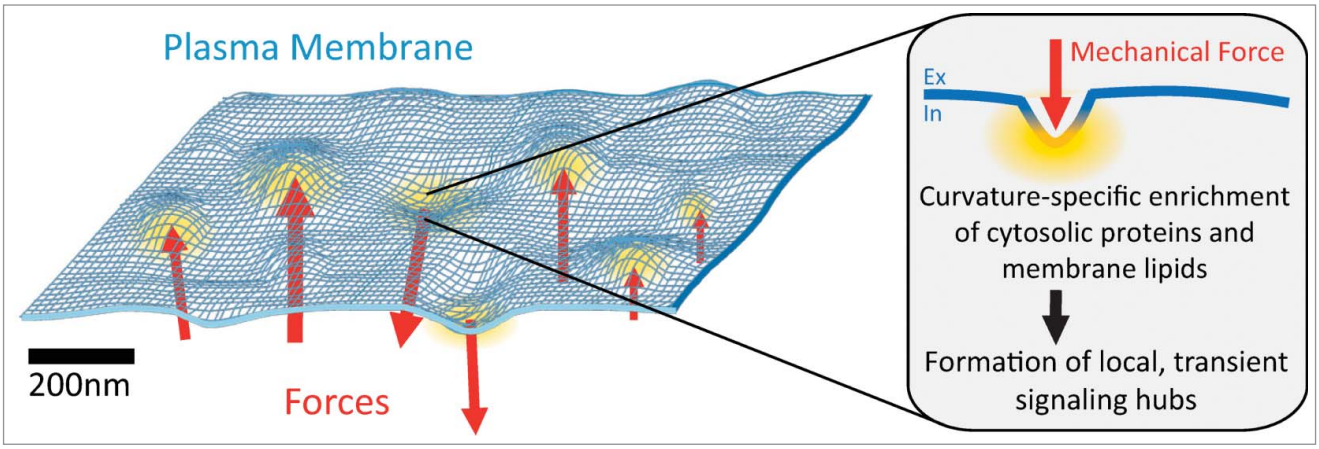

Figure 1. Mechano-chemical signal translation at cellular membranes. Forces (red arrows) applied to the cell cause inward and outward plasma membrane deformations. These deformations trigger enrichment of curvature-sensing cytosolic proteins and lipids (yellow), which form transient signaling hubs critically involved in cell signaling and force-control.

these proteins work in ensembles. Forces generated by single molecules range between 5 and 10 piconewton,[3,4] which, if applied to the PM, is not sufficient to cause deformations of the membrane that can be detected by curvature-sensing proteins.[15] The requirement of multiple proteins to generate the force required to deform the membrane not only prevents puncturing of the plasma membrane or accidental initiation of curvature-dependent mechano-chemical signal translation, but also provides the possibility of forming complex signaling and regulatory mechanisms, on which we will focus in this commentary.

What molecules are recruited to curved membranes, has been the topic of many excellent reviews (reviewed in [17-22]), and will only be briefly discussed. Dozens of proteins with various function sense membrane curvature either as monomers and oligomers, or as protein polymers, and enrich at bent membranes.[15,23,24] Furthermore, several lipid species have been shown to reorganize within the plasma membrane in a curvature-dependent manner,[8,9] likely increasing binding affinity of lipid-binding proteins[25,26] to curved membranes.

Force-Regulating Feedback Loops

Probably the best studied process involving plasma membrane deformation is the highly choreographed sequential recruitment of individual proteins during Clathrin-mediated endocytosis. Here, initial assembly steps are coordinated at least in part by membrane curvature.[27] More recently, a second group of curvature-sensitive proteins linked to actin polymerization dynamics has emerged. This group includes among others the BAR domain proteins Oligophrenin that has been linked to fragile X syndrome,[28,29] srGAP2 that has been shown to be pivotal for migration and maturation of neuronal progenitors during cortex development [30,31] and srGAP3 that has been linked to mental retardation.[32]

Curiously, and despite the fact that endocytosis and actin dynamics are different mechanisms, many of the proteins involved in these 2 processes not only sense but are also capable of inducing membrane deformation. This observation that proteins not only respond to but also elicit mechanical deformations of the PM, argues for the existence of force-regulating feedback loops. In theory, such a feedback can either rely on a dual function of proteins capable of sensing curvature and deforming membranes, or proteins may regulate the activity of membrane-deforming protein-polymers that *per se* do not show curvature-dependence. One example for such an indirect type of force control is ArhGAP44.[33] This positive curvature-sensor (i.e. inward PM deformation) contains a GAP domain that is directed against the small GTPase Rac1 and Cdc42. In neurons, recruitment of this protein to plasma membrane deformations creates a negative feedback loop that limits actin dynamics at nascent filopodia and aborts initiation of exploratory dendritic filopodia.[33] The second example, Baiap2, is in many ways the complementary example to ArhGAP44. This protein acts as a negative curvature-sensor (i.e., outward PM deformation), which creates a positive feedback loop via the recruitment of adaptor proteins that augments actin-dynamics and filopodia formation.[34]

How widespread is this force-regulating principle? The variability in curvature-preference and selectivity for targeted actin-regulatory enzymes, and the large number of proteins capable of forming such curvature-dependent feedback loops, suggests that cells may use this mechanism to control a broad spectrum of actin-dependent processes. It is thus plausible to assume that the spatio-temporal actin dynamics, and in consequence the forces that shape cell architecture, are controlled at least partially by curvature-dependent mechano-chemical signal translation.

Technical challenges and advancements

Approaches to study curvature-dependent properties of proteins include crystal structure analysis,[11] binding of curvature-sensing proteins to vesicles of different diameters,[15] or tubulation of lipid vesicles.[11] In these assays, proteins are probed for their ability to sense or induce

membrane curvature *in vitro*. However, considering that the PM lipid composition of the inner leaflet is still not well known, these approaches do not show whether recruitment of curvature-sensing proteins is selective to particular curved membranes within the cell. Furthermore, dynamic measurements of curvature-sensing proteins in cells suggest that many of the relevant membrane binding events are short lived and selective to particular lipids within the PM.[27] *In vitro* approaches do not provide dynamic insights into how curvature-sensing proteins assemble and disassemble in their physiological setting (many curvature-sensing proteins form oligomers when they bind to curved membranes). Consequentially, these methods are not well suited to determine if and how individual curvature-sensing proteins dynamically interact or compete when binding to curved plasma membranes.

Why is this important? The fact that proteins that induce membrane deformations are regulated by bent membranes creates a causality dilemma. To answer what the cause and what the consequence of membrane deformations is, new techniques are needed. Recently, such a complementary approach has been introduced that relies on nanomaterials to mimic protein-dependent membrane deformations in living cells.[35,36] Here, cone-shaped nanostructures with a height of 200–600 nm and a tip diameter

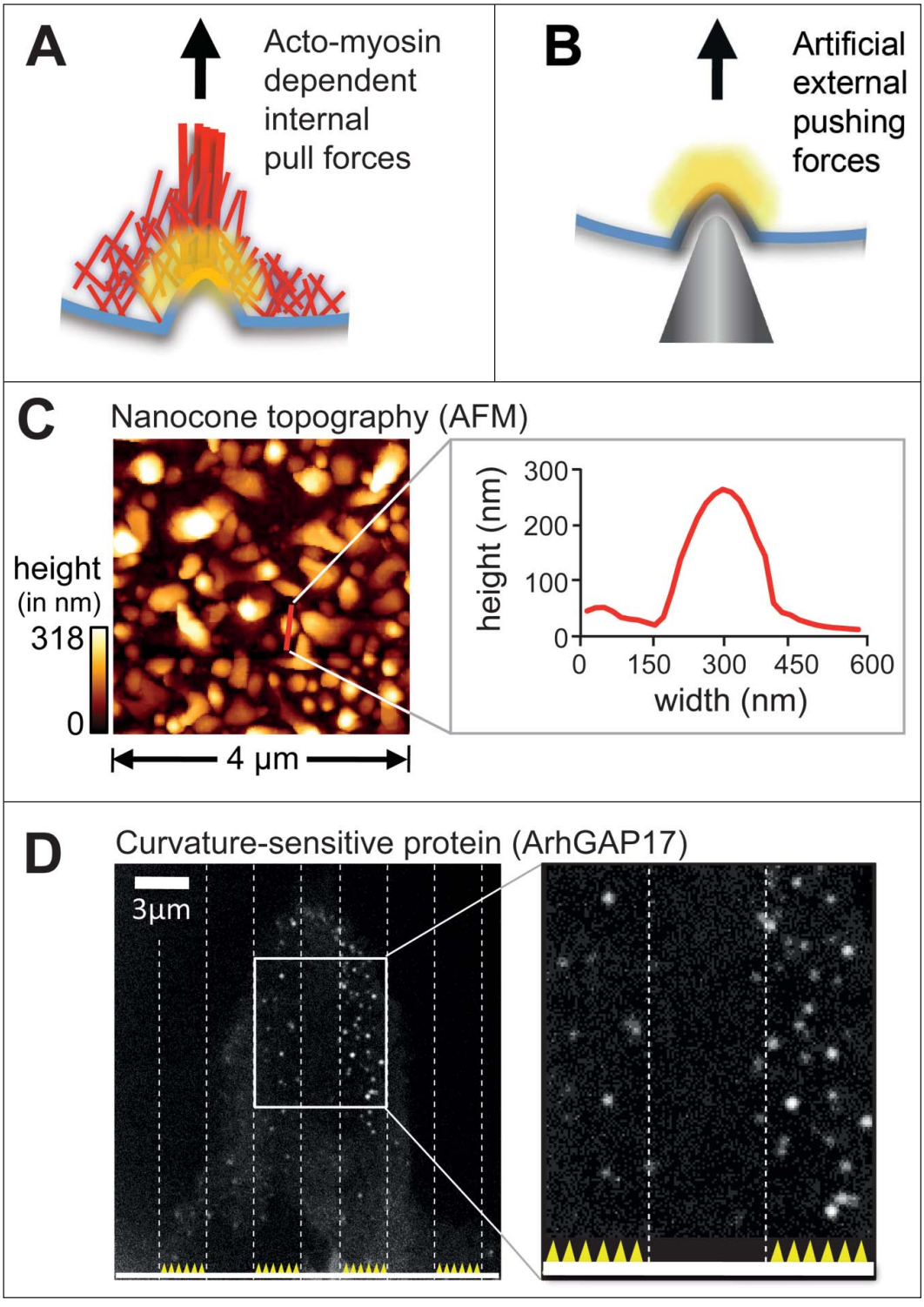

Figure 2. Internal pull and artificial external push forces create plasma membrane deformations. (**A**) Inward plasma membrane deformation by acto-myosin dependent contraction of membrane-associated actin cables. Schematics depicting individual actin filaments (red), as well as lipids and cytosolic proteins, that are recruited in a curvature-dependent manner to curved membranes (yellow). (**B**) Inward plasma membrane deformation created by cone-shaped nanostructures. (**C**) Atomic force microscope image of the surface of cone-shapes nanostructures. The height profile of one nanocone (red line) is shown to the right. (**D**) Selective recruitment of the curvature-sensitive N-BAR domain containing protein ArhGAP17 to nanocone-induced membrane deformations in live cells. Note that nanocones are deposited in a striped pattern (yellow triangles).

of ~50 nm were used to indent the plasma membrane of cells cultured on such a substrate (**Fig. 2**). This approach allowed for the first time to investigate curvature-dependent protein recruitment to the plasma membrane under physiological conditions (i.e. lipid asymmetry of the membrane bilayer, presence of integral membrane-proteins, correct pH, osmolarity, etc.). More importantly, however, as membrane deformation was in this case not triggered by protein polymers but artificially induced via nanostructures, it allowed delineating curvature-dependent protein recruitment from events that otherwise would occur at the same time.

Alas, since membrane deformation in this setup relies on passive indentation of the plasma membrane of cells migrating over these nanostructures, onset and amplitude of the membrane deformation is not controllable in this system. Thus, to investigate kinetic (e.g., on and off rates) and spatial aspects (curvature-preference) of protein recruitment in living cells, other tools need to be developed.

Open questions

If other cytoskeletal protein polymers, such as microtubules or intermediate filaments, are subject to such curvature-dependent force control remains elusive. However, it is in this context worth mentioning, that recent work showed that polarized microtubules can be associated with the plasma membrane, thus providing a functional linkage to the membrane.[37] It is thus plausible to envision that force-regulating feedback loops that regulate spatio-temporal dynamics of cytoskeletal proteins at curved membranes may reflect a general signaling principle cells use to regulate cellular forces in a receptor independent manner.

While these recent advancements argue for curvature-dependent force-regulating feedback loops as a new form of mechanochemical signal translation, the function of individual lipids in this process has largely remained unclear. Lipid composition determines rigidity and fluidity of membranes. Consequentially, changes in the concentration of individual lipids not only alter membrane composition, but also the force required to deform the membrane and the time that is required to enrich specific lipids at such curved sites (i.e., membrane viscosity). Examples where this may be relevant include among others aging and lipid-based disease, where changes in the lipid composition of the plasma membrane have been reported.[38-40] It is feasible to envision that changes in force-dependent lipid signaling are altered in aging and disease conditions. However, only future work using new biosensors to monitor lipid reorganization[41,42] and force-generation,[43-45] will allow us to monitor lipid-dependence of mechano-chemical signal translation, and provide insights into how the topographical distribution of individual lipid species is affected by age- and disease-dependent changes in membrane composition.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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