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.**

**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 in1,2), 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 intracellular3,4 and intercellular5-7 forces. In response to such nanoscale membrane deformations, cytosolic proteins and membrane lipids are enriched in a curvature-dependent manner8-11. Since some of the recruited molecules contain signaling properties12-14, 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 selectivity15, 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:
these proteins work in ensembles. Forces generated by single molecules range between 5 and 10 piconewton, which, if applied to the PM, is not sufficient to cause deformations of the membrane that can be detected by curvature-sensing proteins. 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), 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. Furthermore, several lipid species have been shown to reorganize within the plasma membrane in a curvature-dependent manner, likely increasing binding affinity of lipid-binding proteins 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. 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, srGAP2 that has been shown to be pivotal for migration and maturation of neuronal progenitors during cortex development and srGAP3 that has been linked to mental retardation.

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. 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. 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 adapter proteins that augments actin-dynamics and filopodia formation.

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, binding of curvature-sensing proteins to vesicles of different diameters, or tubulation of lipid vesicles. In these assays, proteins are probed for their ability to sense or induce...
membrane curvature \textit{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.\textsuperscript{27} \textit{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). Consequently, 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.\textsuperscript{35,36} Here, cone-shaped nanostructures with a height of 200–600 nm and a tip diameter

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\caption{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).}
\end{figure}
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 used 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 mechano-chemical signal translation, the function of individual lipids in this process has largely remained unclear. Lipid composition determines rigidity and fluidity of membranes. Consequently, 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 reorganization41,42 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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References
1. Leckband DE, le Duc Q, Wang N, de Rooij J. Mecha-
notransduction at cadherin-mediated adhesions. Cur-
rent Opinion in Cell Biology 2011; 23:523-30; PMID:21890337; http://dx.doi.org/10.1016/j.celb.2011.08.003
2. Vogel V. Mechanotransduction involving multimodu-
lar proteins: converting force into biochemical signals. Annual Review of Biophysics and Biomolecular Structure 2006; 35:459-88; PMID:16689645; http://dx.doi.org/10.1146/annurev.biophysics.35.040405.102013
3. Peskin CS, Ostdahl GM, Oster GF. Cellular motions and thermal fluctuations: the Brownian ratchet. Biophysical Journal 1993; 65:316-24; PMID:8369439; http://dx.doi.org/10.1016/0006-3495(93)81035-X
4. Finer JT, Simmons RM, Spudich JA. Single myosin mole-
cule mechanics: piconewton forces and nanometer steps. Nature 1994; 368:113-9; PMID:8319653; http://dx.doi.org/10.1038/368113a0
5. le Duc Q, Shi Q, Blonk I, Sonnenberg A, Wang N, Leckband D, de Rooij J. Vinculin potentiates E-cad-
herin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. The Journal of Cell Biology 2010; 189:107-15; PMID:20584916; http://dx.doi.org/10.1083/jcb.201001149
6. Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. Scien-
tice 1993; 260:1124-7; PMID:7684161; http://dx.doi.org/10.1126/science.260.5116.1124
7. Ng MR, Besser A, Brugge JS, Dausser G. Mapping the dynamics of force transduction at cell-cell junctions of epithelial clusters. eLife 2014; 3; PMID:25479385; http://dx.doi.org/10.7554/eLife.03282
8. Wang W, Yang L, Huang HW. Evidence of cholesterol accumulated in high curvature regions: implication to the curvature elastic energy for lipid mixtures. Biophysical Journal 2007; 92:2819-30; PMID:17259720; http://dx.doi.org/10.1529/biophysj.106.097923
9. James DJ, Khododong C, Kowalczyk JA, Martin TF. Phosphatidylinositol 4,5-bisphosphate regulates SNARe-dependent membrane fusion. The Journal of Cell Biology 2008; 182:355-66; PMID:18648490; http://dx.doi.org/10.1083/jcb.200801056
10. Habermann B. The BAR-domain family of proteins: a case of bending and binding? EMBO Reports 2004; 5:250-5; PMID:14993925; http://dx.doi.org/10.1038/sj.embor.7400105
11. Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT. BAR domains as sensors of mem-
brane curvature: the amphiphysin BAR structure. Science 2004; 303:495-9; PMID:14645856; http://dx.doi.org/10.1126/science.1092586
12. Head BP, Patel HH, Insel PA. Interaction of mem-
brane/lipid rafts with the cytoskeleton: impact on sig-
naling and function: membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. Biochi-
mica et Biophysica Acta 2010; 1806:332-45; PMID:20389502; http://dx.doi.org/10.1016/j.bbagen.2013.07.018
13. Sechi AS, Wehland J. The actin cytoskeleton and plasma membrane connection: PtdIns(4,5)(P)2 influ-
ences cytoskeletal protein activity at the plasma mem-
brane. Journal of Cell Science 2000; 113 Pt 21:3685-
95; PMID:11034897
14. Nie Z, Hirsh DS, Luo R, Jian X, Stauffer S, Cremesti A, Andrade J, Lebowier J, Mazino M, Alvarez B, et al. A BAR domain in the N terminus of the Arf GAP ASAPI affects membrane structure and trafficking of epidermal growth factor receptor. Current Biology : CB 2006; 16:130-9; PMID:16431655; http://dx.doi.org/10.1016/j.cub.2005.11.069
15. Bhathia VK, Madsen KL, Bolinger PY, Kunding A, Hedegaard P, Gerber U, Stamos D. Amphipathic motifs in BAR domains are essential for membrane curvature sensing. The EMBO Journal 2009; 28:3505-14; PMID:19816046; http://dx.doi.org/10.1038/embj.2009.261
16. Mathila PK, Pykalainen A, Saarkivuori J, Paavilainen VO, Vihinen H, Jokitalo E, Lappalainen P. Missing-in-
metastasis and IRS5P3 deform Ptd(4,5)P2-rich mem-
branes by an inverse BAR domain-like mechanism. The Journal of Cell Biology 2007; 176:953-64; PMID:17371834; http://dx.doi.org/10.1083/jcb.200609176
17. Kozlov MM, Campello F, Liska N, Chermokid LV, Martijn SI, McMahon HT. Mechanisms shaping cell mem-
branes. Current Opinion in Cell Biology 2014; 29:53-60; PMID:24747171; http://dx.doi.org/10.1016/j.cceb.2013.05.006
18. Schnick M, Bastaens PI. The interdependence of membrane shape and cellular signal processing. Cell 2014; 156:1132-8; PMID:24630771; http://dx.doi.org/10.1016/j.cell.2014.02.007
19. Bigay J, Antony B. Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular ter-
itories in determining specificity. Developmental Cell 2012; 23:886-95; PMID:22315348; http://dx.doi.org/10.1016/j.devcel.2012.10.009
20. Antony B. Mechanisms of membrane curvature sens-
ing. Annual Review of Biochemistry 2011; 80:101-3; PMID:21436688
21. McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 2005; 438:590-6; PMID:16319878; http://dx.doi.org/10.1038/nature04398
22. Drin G, Antony B. Amphipathic helices and mem-
brane curvature. FEBS Letters 2010; 584:1840-7; PMID:19837060; http://dx.doi.org/10.1016/j.febslet.2009.10.022
23. Bigay J, Casella JF, Drin G, Mesmin B, Antony B. ArfGAP1 responds to membrane curvature through the
folding of a lipid packing sensor motif. The EMBO Journal 2005; 24:2244-53; PMID:15949734; http://dx.doi.org/10.1038/sj.emboj.7600714

24. Rothman JE. Life without clathrin. Nature 1986; 319:96-7; PMID:3079886; http://dx.doi.org/10.1038/31906b0

25. Harlan JE, Hajduk PJ, Yoon HS, Fesik SW. Pleckstrin homology domains bind to phosphatidylinositol-4,5-biphosphate. Nature 1994; 371:68-70; PMID:8072546; http://dx.doi.org/10.1038/371168a0

26. Ono Y, Fujii T, Igarashi K, Ueno T, Tanaka C, Kikawa U, Nishizuka Y. Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. Proceedings of the National Academy of Sciences of the United States of America 1989; 86:4868-71; PMID:25200657; http://dx.doi.org/10.1073/pnas.86.13.4868

27. Taylor MJ, Perrais D, Merrifield CJ. A high precision survey of the molecular dynamics of clathrin-mediated endocytosis. PLoS Biology 2011; 9: e1000604; PMID:21445324; http://dx.doi.org/10.1371/journal.pbio.1000604

28. Govek EE, Newey SE, Akerman CJ, Cross JR, Van der Sandhoff K, Polyakova S, Belov VN, Hein B, von Middendorff C, Schonle A, et al. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 2009; 457:1159-62; PMID:19098897; http://dx.doi.org/10.1038/nature07596

29. Johnson HW, Schell MJ. Neuronal IP3 3-kinase is an F-actin-bundling protein: role in dendritic targeting and regulation of spine morphology. Molecular Biology of the Cell 2009; 20:5166-80; PMID:19846664; http://dx.doi.org/10.1091/mbc.E09-01-0083

30. Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neu-kirchen D, Bista M, Bradle F, Jenne D, Holak TA, Werb Z, et al. Lifeact: a versatile marker to visualize F-actin. Nature Methods 2008; 5:605-7; PMID:18536722; http://dx.doi.org/10.1038/nmeth.1220

31. Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, Jin WL, Vanderhaeghen P, Ghosh A, Sassa T, et al. Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. Cell 2012; 149:923-35; PMID:22559944; http://dx.doi.org/10.1016/j.cell.2012.03.034

32. Endrino V, Wagetzky B, Leimner U, Bartisch D, Zaryka M, Latif F, Maher ER, Tarverdian G, Kirsch S, Karch D, et al. The novel Rho-GTPase activating gene MEGAP2 srGAP2 has a putative role in severe mental retardation. Proc Natl Acad Sci U S A 2002; 99:11754-9; PMID:12195014; http://dx.doi.org/10.1073/pnas.162241099

33. Galic M, Tsai FC, Collins SR, Matis M, Bandara S, Meyer T. Dynamic recruitment of the curvature-sensitive protein ArhGAP44 to nanoscale membrane deformations limits exploratory filopodia initiation in neurons. eLife 2014; 3: http://dx.doi.org/10.7554/eLife.03116

34. Krugmann S, Jordens I, Gevaert K, Driessens M, Vandekerckhove J, Hall A. Cdc42 induces filopodia by promoting the formation of an IRSp53:Mena complex. Current Biology: CB 2001; 11:1645-55; PMID:11696321; http://dx.doi.org/10.1016/S0960-9822(01)00506-1

35. Galic M, Jeong S, Tsai FC, Joubert LM, Wu YI, Hahn KM, Cui Y, Meyer T. External push and internal pull forces recruit curvature-sensing N-BAR domain proteins to the plasma membrane. Nature Cell Biology 2012; 14:874-81; PMID:22750946; http://dx.doi.org/10.1038/nclb2533

36. Jeong S, Galic M. Nanocoes to study initial steps of endocytosis. Methods in Molecular Biology 2014; 1174:275-84; PMID:24947389; http://dx.doi.org/10.1007/978-1-4398-0944-5_19

37. Mati M, Ruster-Germain DA, Hu Q, Tomlin CJ, Axelrod JD. Micromotutes provide directional information for core PCP function. eLife 2014; 3:e02893; PMID:25124458; http://dx.doi.org/10.7554/eLife.02893

38. Karten B, Vance DE, Campenot RB, Vance JE. Cholesterol accumulates in cell bodies, but is decreased in distal axons, of Niemann-Pick C1-deficient neurons. Journal of Neurochemistry 2002; 83:1154-63; PMID:12437588; http://dx.doi.org/10.1006/jncl.2002.11200

39. Ledesma MD, Martín MG, Domínguez MC. Receptor-induced translocation of the raf-1 mitogen-activated protein kinase 1 in human endothelial cells. J Cell Sci 1998; 111:401-7; PMID:9658681; http://dx.doi.org/10.1042/jcs1110401

40. Arroyo AI, Camoletto PG, Morandi L, Sasso-Poggetto M, Giustetto M, Van Veldhoven PP, Schuchman EH, Ledesma MD. Pharmacological reversion of sphingomyelin-induced dendritic spine anomalies in a Niemann-Pick disease type A mouse model. EMBO Molecular Medicine 2014; 6:398-413; PMID:24448491