Mechanobiology — chemical origin of membrane mechanical resistance and force-dependent signaling
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The cell membrane is a highly complex designed material with remarkable physicochemical properties; comprised mainly of lipid moieties, it is capable of self-assembling, changing morphology, housing a range of distinct proteins, and withstandstanding electrical, chemical and mechanical perturbations. All of these fundamental cellular functions occurring within a 5 nm thick film is an astonishing feat of engineering, made possible due to the interplay of a variety of intermolecular forces. Elucidating how the interactions within the chemically distinct partners influence the nanomechanical properties of the membrane is essential to gain a comprehensive understanding of a wide-variety of both force-triggered and force-sensing mechanisms that dictate essential cellular processes.

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Current Opinion in Chemical Biology 2015, 29:87–93
This review comes from a themed issue on Mechanistic Biology
Edited by Paula Booth and Lynne Reagan

http://dx.doi.org/10.1016/j.cbpa.2015.09.019
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Introduction
Mechanotransduction defines a plethora of molecular processes that cells adopt to translate mechanical stimuli into biochemical signals [1], which determine a variety of cell functions encompassing motility [2], cell shape, proliferation [3], differentiation [4,5], development [6], tumorigenesis [7] and wound healing [8]. In general, a wide variety of molecular players have been described to participate in the transmission of mechanical forces from the extracellular matrix that are channeled over the cytoskeleton to eventually reach the cell nucleus [9]. In this context, cellular mechanosensing has been mostly tackled from the perspective of force-induced conformational changes in mechanosensitive proteins, such as integrins, or the focal-adhesion partners α-actinin, talin or vinculin, which stimulate downstream signaling cascades [10]. Despite such tantalizing progress, the mechanical role of the lipid membrane, which has often been merely regarded as a physical pseudo-elastic barrier, has been comparatively less studied.

The chemical complexity of cellular membranes
The fascinating lipid complexity of the eukaryotic cell, containing thousands of individual lipids synthesized using 5% of its genes [11], sharply contrasts with the simplistic view of the membrane being an inert wall. Lipids allow membranes to undergo massive conformational changes, such as budding, tubulation, fission or fusion, which are required for capital cell functions such as endocytosis and exocytosis, membrane trafficking and cell division [11]. Such strong mechanical requirements are only possible due to a fine-tuned regulation of the chemical composition of the lipid membrane, which in turn determines its physicochemical properties. Despite the limited knowledge of the lipidome of most cells, some important features are already emerging. As it is schematically shown in Figure 1, it is by now clear that different lipid species are non-randomly distributed between subcellular organelles [12], and that the chemical composition of the membrane of each organelle is intimately linked to its function [11]. For example, while the plasma membrane is enriched in highly packed sphingolipids with straight saturated acyl chains and high sterol concentration to resist mechanical stress, the cis-Golgi and the ER — which is the main lipid biosynthetic reservoir — are rich in ‘kinked’ monounsaturated chains that form loosely packed membranes that contain defects in the geometrical arrangement of their lipids, consistent with their function of facilitating the transport of newly synthesized lipids [11–13]. Further increasing the level of unsaturation of the fatty acid chains endows polyunsaturated-rich lipid membranes with particular mechanical properties with enhanced deformation capabilities, favoring for example rapid endocytosis [14]. Such increased membrane flexibility plays an important role in the functioning of synaptic vesicles and mechanoreceptor neurons [15].

In sharp contrast with the huge heterogeneity in the cellular lipid composition, three simple intertwined physicochemical parameters, namely membrane charge density, membrane curvature and lipid packing, seem
sufficient rough estimators to explain and predict the physical remodeling and shape of several organelles [13]. Membrane electrostatics largely depends on the negatively charged phosphatidylerine (PS), mostly present in the cytosolic side of the plasma membrane and also in the luminal side of the ER, and phosphoinositide phosphatidylinositol(4,5)bisphosphate (PIP2), which maintains cell shape by linking the cytoskeleton actin cortex to the membrane [16,17] and is also localized in the inner leaflet of the plasma membrane. On the other hand, lipids with unsaturated acyl chains and/or small headgroups mostly foster packing defects, which enhance membrane curvature. On the basis of these considerations, the cell can be divided into two main regions with markedly different membrane properties; in the ER and in the cis-Golgi, membranes are poorly charged in their cytosolic leaflet and exhibit packing defects due to their high concentration of lipids with monounsaturated chains. By contrast, membranes from the trans-Golgi to the plasma membrane display negative lipids on their cytosolic side and are tightly packed as a result of their saturated lipids [13]. These simple considerations already highlight a close correlation between chemical composition and lipid packing, which ultimately dictates the mechanical architecture of the membrane, largely defining organelle functionality.

**Mechanical probing of membranes**

Several experimental techniques, mostly based on light and force microscopies, have been employed to directly quantify the changes in membrane morphology and the mechanical forces required to withstand the massive membrane rearrangements occurring during the course of a variety of cellular activities (e.g. cell migration and endocytosis and exocytosis) [18]. In particular, the deformation of pillar arrays, traction force microscopy and optical and magnetic tweezers have provided a quantitative view on the deformability of individual [19], and also concatenated cells monolayers [20], when exposed to a stretching, pulling force. In all these in *vitro* approaches, however, the regulation of membrane dynamics is intricately coupled with the actin cytoskeleton and related contractile structures [18,21], in such a way that it is challenging to decipher the mechanical role of the membrane alone [22].

To investigate *in vitro* the mechanical properties of membranes, and to gain understanding on how subtle changes in their chemical composition affect the overall mechanical response, the micropipette aspiration technique paved the way for quantifying, at the mesoscopic scale, the elastic properties of giant lipid vesicles [23,24]. However, due to the mosaic nature of biological membranes, often featuring submicroscopic domains, other localized techniques exploring the nanometer realm such as force spectroscopy with atomic force microscopy (AFM) have been progressively used [25]. In these experiments, the tip of the AFM cantilever is typically used as a nanoneedle force sensor that penetrates an individually supported bilayer [26]. The compressing

The complexity in the lipid composition of the different membranes within the cell regulates the shape and function of the distinct organelles.

**Figure 1**
force required to indent the bilayer fingerprints the mechanical stability of the membrane, as shown in Figure 2a (red arrow) [27]. This approach has been used to determine how subtle changes in the chemical composition of the phospholipid membranes, involving both the headgroup and the hydrophobic tail, have a large impact on the overall mechanical resistance [28]. The local physicochemical environment of the bilayer, namely ionic strength [29] and temperature [30], has also been demonstrated to play an important role on the mechanical behavior of the membrane. Despite such progress, this approach has two intrinsic limitations; firstly the supported membranes are confined to two dimensions, thus impeding measurement of the membrane’s intrinsic curvature, and secondly, the measured mechanical properties cannot be unambiguously decoupled from the rigid supporting substrate.

To circumvent these limitations, pore spanning bilayers, where free-standing membrane patches are spread across a substrate containing an array of holes, permit decoupling of the substrate effect while allowing measurement of membrane bending and stretching [31]. An alternative emerging approach relies on studying the mechanical properties of stacked lipid bilayers, where the individual rupture of up to a hundred layers is followed, one at a time (Figure 2b). These novel experiments, while also avoiding any contribution from the stiff supporting substrate, allow measurement of the force-dependent kinetics of the rupturing process. The stacked lipid approach combined with functionalized tips was used as a platform to mimic the interfacial mechanisms underlying the insertion of membrane proteins into the hydrophobic core of the membrane [32,33,34∗]. On a larger scale, it also allows investigation of the interlamellar interactions, revealing for example that different phase domains align over time in the 3D vertical direction [35]. From a more generic perspective, the multibilayer approach provides an in vitro means to study the nanomechanical properties of organelles featuring more than a single bilayer, such as the case in mitochondria or the nuclear envelope.

All this progress notwithstanding, the abovementioned methods are, by definition, restricted to study the nanomechanical properties of membranes in vitro. AFM experiments have also been widely expanded to study the mechanical properties of individual live cells [36]. When low compressing (pushing) forces are applied on the cell surface by the AFM cantilever tip, the elastic properties of the cell can be readily extracted upon the application of pertinent mechanical models [37,38]. Again, in these experiments the overall measured elastic response stems from a combined contribution of the plasma membrane and the underlying cytoplasm and cytoskeleton [39]. Applying a higher force to the individual cell probes the mechanical properties of the entire range of cellular compartments. In particular, these experiments recently demonstrated that the mechanical properties of cell membranes can be independently measured in the context of those

![Figure 2](image-url)

Probing the mechanical properties of (a) supported, (b) multi-stacked and (c) native membranes using force spectroscopy AFM.
whole-cell experiments [40,41*]. In these cases, the mechanical stability of the plasma membrane can be fingerprinted by two consecutive ~5 nm jumps in the force versus distance trajectories, hallmarking the indentation of the AFM tip through the outer and inner plasma membrane, respectively, as the AFM tip squeezes cytoplasmic region of the cell against the solid substrate. Analogously, when the cell is indented traversing the nucleus, up to 4 extra indentation jumps are measured, corresponding to the puncturing of the two double membrane composing the nuclear envelope (Figure 2c). Surprisingly, the forces required to puncture these membranes in live cells closely correlate with those measured in vitro, suggesting that those in vitro assays provide a first good physiological representation of membrane nanomechanics [41*].

Membranes as 'indirect' transducers of mechanical forces. Membrane tension can be sensed and promoted by several proteins (a), and is able to activate gating in a variety of mechanosensitive ion channels (b). The plasma membranes serve as a support for integrins and focal adhesions, which are crucial in the transmission of mechanical pathways from the ECM to the cytoskeleton.
**Distinct mechanical roles of cellular membranes**

In spite of such progress, the complexity in the molecular origin of the multiple mechanical roles of membranes in vivo poses experimental challenges that still remain partially unresolved. Beyond its ‘direct’ role of acting as a stiff mechanical accumulating evidence highlights that membranes serve as ‘indirect’ dynamic platforms for localization of various molecular players that actively participate in all aspects of the motility process, including force generation, adhesion and signaling. In this context, the concept of membrane tension, largely changing the curvature and shape of the membranes of cellular organelles, emerges [21]. Effective tension in cells is a consequence of two factors, namely the in-plane tension due to hydrostatic pressure difference across the membrane and the adhesion of the membrane to the cytoskeleton, mediated by phospholipid-binding proteins [42,43]. Although the relative contribution of both players might vary, it is clear that surface tension has an important influence on membrane remodeling and the resulting cellular morphology [18]. In order to generate curvature, proteins can either make the lipid bilayer asymmetric, or apply forces to the membrane surface resulting in membrane bending [44]. Examples of the latter group include the Bin-ampiphysin-Rvs (BAR) superfamily proteins [45], dynamin and epsin, which induce membrane curvature both in vitro and in cells, hence connecting direct membrane deformation to actin polymerization during morphogenesis. The membrane curvature sensing mechanism (scaffolding) employed by BAR proteins [46], displaying a concave surface decorated with cationic residues that adsorb preferentially onto ionic lipids of convex membranes [47,48], contrasts with the sensing mechanisms used by ALPS motifs, that rather than sensing surface geometry per se, they target defects in lipid packing arising from the deformation of the bilayer [49**]. Both strategies are schematically illustrated in Figure 3a. These intrinsically unfolded motifs of ALPS, harbouring amphipathic sequences, use their bulky hydrophobic residues to detect lipid-packing defects caused by the mismatch between the curvature of the membrane and the spontaneous curvature of its cytosolic leaflet [50*]. Irrespective of these used mechanisms, the mechanics of the membrane, in the form of membrane tension and curvature, acts in all these cases as an ‘indirect’ recruiting agent for proteins that synergize with membrane composition to facilitate the dynamic behavior that regulates the shape of cellular membranes [51,52].

Another class of secondary or ‘indirect’ mechanism by which membrane tension regulates cellular behavior is the activation of mechanosensitive ion channels. For a channel to be mechanosensitive, mechanical stresses need to change the channel conformation to stabilize either the open or the closed state [53]. Both in the bacterial mechanosensitive channels MscL and MscS [54] and also in the eukaryotic TRAAK and TREK1K* ion channels [55**,56] the mechanical forces gating the channels are directly transmitted from the bilayer to the channel [57], through a lateral membrane tension mechanism (Figure 3b).

Finally, a certainly more passive, yet still seemingly important role of lipids in mechanotransduction lies in its physical support for transmembrane integrins (Figure 3c), which connect the extracellular matrix (ECM) to the cell cytoskeleton [58]. Likewise, external mechanical forces can be transmitted to the cell interior through a complex and dynamic network of adaptor proteins forming the focal adhesions [10]. Transmission of these mechanical signals through the plasma membrane, which can eventually reach the nuclear envelope [9], largely determine cell behavior and fate.

**Concluding remarks**

Starring a wide spectrum of roles, lipid membranes are rapidly emerging as key players in the transduction of mechanical cues to and within the cell. Either through the direct role as a mechanical scaffold — finely regulated by the chemical composition of the lipid moieties — , or through a more subtle, indirect performance by bending membranes or creating perfectly designed nanometer-scale defects, membranes control a wide variety of mechanosensitive processes that are crucial to cell functioning, the failure of these entailing dire consequences [59]. Despite its importance, and often due to the single molecule ethos of these processes, very few experimental tools are available to relate, in vivo, the complex interplay between the membrane chemical composition and its physical, mechanical role. A further layer of complexity lies in the fast dynamics of membranes [60]. Rather than being a static entity, new evidences suggest that the lipidome is dynamically changing with for example the cell cycle, with 11 lipids with specific chemical structures accumulating in dividing cells. Crucially, the active up-regulation of these lipids results in a drastic change in the mechanical properties of the cell. These observations demonstrated that cells actively regulate their lipid composition and localization during division, with both signaling and structural roles alike [41*].

Despite these technical difficulties, several complementarity in vitro experimental probes, combined with computer simulations, are building up an integrated, physiological representation of membrane mechanics. Taken together, these studies recognize lipid membranes dynamic regulators of a variety of cellular processes that are triggered by mechanical forces. Because of their large biological implications, these studies are rapidly expanding within the framework of a largely multidisciplinary field.

**Acknowledgements**

AEMB is funded by an EPSRC DTP fellowship. AW is funded by a British Heart Foundation doctoral fellowship. JR-G was recipient of a ‘La Caixa’
fellowship. This work was supported by the Marie Curie CIG (293462) grant, BBSRC (J08992X/1) grant, and EPSRC Fellowship (K00641X/1) to NG-M.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


In this paper, the authors demonstrated that, when phospholipids with polysaturated fatty acid chains were incorporated into cells, their plasma membrane was more deformable under the effect of a pulling force, increasing the rate of endocytosis. This work highlights the direct role of phospholipid composition on the mechanics of cell membrane, greatly affecting its functionality.


Performing AFM-based indentation experiments, the authors compared the mechanical resistance of live cells and stacks of synthetic lipid bilayers. Examination of the penetration force under various mechanical and chemical conditions revealed that the limiting factor for probe insertion into a cell is the rupture of the lipid bilayer itself, and not any other cell-specific component of the membrane.


Using a combination of LC–MS, RNAi knockdown and force spectroscopy AFM, the authors demonstrated that cells specifically regulate the localization of lipids to midbodies during cell division. These experiments revealed that 11 lipids accumulate in dividing cells, dramatically affecting the mechanical properties of their membranes.


Within a combined in vivo, in vitro and in silico approach, the authors showed how curvature and lipid composition are linked in biological membranes in order to direct the recruitment of peripheral proteins to cellular organelles. Their findings demonstrate that membrane curvature and lipid composition have, in the cellular context, cumulative effects.


Using a combination of light microscopy and biophysical techniques, the authors demonstrated the mechanism by which nuclear pore complexes (NPCs) are embedded in the nuclear envelope. Their experiments showed that the NPC basket proteins induce membrane curvature by amphipathic helix insertion into the lipid bilayer.


Using a whole-cell voltage clamp approach, the authors demonstrated the fundamental mechanisms of force transduction in eukaryotic mechanosensitive ion channels. The authors discovered that the mechanical force required to activate the TRAAK and TREK1 K+ channels were transmitted directly from the membrane to the channel, and not through macromolecular tethers attached to the channel.


