Review

Use the force: membrane tension as an organizer of cell shape and motility

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Many cell phenomena that involve shape changes are affected by the intrinsic deformability of the plasma membrane (PM). Far from being a passive participant, the PM is now known to physically, as well as biochemically, influence cell processes ranging from vesicle trafficking to actin assembly. Here we review current understanding of how changes in PM tension regulate cell shape and movement, as well as how cells sense PM tension.

Introduction

One way that cells interact with the world around them is biochemically. For example, binding of soluble extracellular ligands to receptors on the cell membrane can trigger intracellular signaling cascades. More recently, it has become clear that physical interactions are also an important currency of information transfer in cells and tissues [1,2] (reviewed in [3]). In particular, tension in the PM has been shown to regulate many cell behaviors, including vesicle trafficking [4] and cell motility [5,6]. The PM is often described by the fluid mosaic model [7], which characterizes it as a 2D continuous fluid bilayer of lipids with freely diffusing embedded proteins. In this paradigm, the bilayer is considered a uniform semipermeable barrier that serves as a passive matrix for membrane proteins. However, this model is incomplete; lipids are now known to have a much more active role in regulating membrane structure and biological function [8–10] (reviewed in [11]) and the mechanical properties of the PM need to be included for a complete picture.

Mechanically, membranes have a low shear modulus (a result of the fluid nature of the lipid bilayer; $4–10\times10^{-3}$ N/m [12–14]), a high elastic modulus (due to the small stretch in bilayers; 10^3 N/m^2 [15,16]), a variable viscosity (which depends on membrane composition; $0.36–2.1\times10^{-3}$ Pa s for an erythrocyte [16]), and a bending stiffness strongly influenced by membrane proteins and cytoskeletal elements (10^{-19} N m [17–19]). Membrane tension is related to the force needed to deform a membrane. Historically, the term membrane tension has been applied to define different concepts, and this has lead to confusion in the literature. Initially, membrane tension was measured in lipid vesicles (Box 1) in which the force needed to stretch the membrane is the in-plane membrane tension (T_m, N/m). In cells, the force needed to deform the PM is greater than that for a pure lipid vesicle due to contributions from membrane proteins and membrane-to-cortex attachments (MCA) (γ, N/m), which link the membrane to the underlying cortex and also resist membrane deformation. Thus, PM tension, also known as apparent membrane tension or effective membrane tension, is the sum of T_m and γ (Box 2).

Research in recent decades has established the importance of PM tension as a physical regulator of cell motility and morphology [6,20–23], but the mechanism of tension sensation and how membrane tension is integrated in the cell's mechanical properties are unknown. We will focus on how PM tension affects and is affected by other cellular processes and will outline possible mechanisms for membrane tension sensation.

Feedback between PM tension and cellular processes

Some studies point to PM tension being a constant parameter within a given cell type [24]. However, it is unclear whether cells have a preferred 'set point' for PM tension and, if so, how cells measure their PM tension. Moreover, in cells, biological membranes are active in the sense that they are constantly maintained out of equilibrium by cellular processes that contribute to changes in PM area, composition, and MCA protein activity. This adds complexity but also gives the cell multiple routes of adjustment. Several cellular processes affect and are affected by PM tension (Figure 1).

Exocytosis and clathrin-mediated endocytosis

PM tension regulates the balance between exocytosis and endocytosis in numerous systems; exocytosis (which is stimulated by high membrane tension) acts to decrease PM tension, whereas endocytosis (which is stimulated by low membrane tension) increases it [25] (reviewed in [4,24,26,27]). These opposing effects of vesicle trafficking could enable cells to keep tension close to a set point [28]. When the PM reservoir is reduced following cell spreading, there is a twofold increase in PM tension followed by activation of exocytosis and myosin-based contraction [20]. The rate of spreading and the time point at which exocytosis and myosin contraction occur are highly dependent on PM tension. These data implicate tension in coordinating membrane trafficking, actomyosin contraction, and PM area change. More recently, MCA has been shown to determine the actin dependence of clathrin coat

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Box 1. Techniques to measure and manipulate membrane tension

Measuring membrane tension

Experiments to determine mechanical properties of biological membranes began in the 1930s using sea urchin eggs and red blood cells [60,61]. Since then, new higher-resolution techniques have been developed. Here we list the most commonly used approaches for measuring membrane tension.

Compression of the cell with two plates (Figure Ia**) and micropipette aspiration (**Figure Ib**)**. These techniques have been extensively used for studying the mechanical properties of membranes in lipid vesicles, urchin eggs, and red blood cells [15,18,60–63]. However, they are applicable only to lipid vesicles or suspension cells with simple morphologies and cannot be used for cells with complex morphologies such as neurons or neutrophils. Moreover, in cells, isolating the contribution of PM tension to these measurements is complicated by the fact that a significant portion of the measured forces can be due to deformations of the cytoskeleton, in particular the actin cortex that lies immediately under the PM.

Tethers. Tethers (which lack a continuous cytoskeleton) have been studied to measure PM tension. Initially these experiments were performed using a micropipette to hold cells or lipid vesicles and a second pipette to extract a membrane tether (Figure Ic) [64]. More recently atomic force microscope (AFM) cantilevers, optical tweezers, and magnetic tweezers have enabled higher-resolution measurements of PM tension (Figure Id) [65–67]. See Box 2 for an in-depth description of how tethers can be used to measure membrane tension.

Fluorescence resonance energy transfer (FRET)-based biosensors. These were recently developed for assaying tension in the cytoskeleton and at sites of adhesion [68]. Although the field currently lacks comparable imaging-based sensors for membrane tension, such a tool would enable less invasive analysis of the spatial and temporal dynamics of PM tension in living cells. Moreover, intracellular organelles are not accessible for tether experiments, and such technology would allow us to determine whether the PM is the only organelle that can act as a mechanical sensor.

Manipulating membrane tension

Vesicle fusion, lipid addition, and changes in osmolarity. These have been used to manipulate T_m [6,69], but none is quantitative unless combined with simultaneous measurements of tension such as tether pulling. Moreover, how much those techniques affect cytoskeletal components is unknown.

Multiple tethers. Pulling multiple tethers with an AFM [70] is an alternative method that can be used to measure and manipulate membrane tension simultaneously.

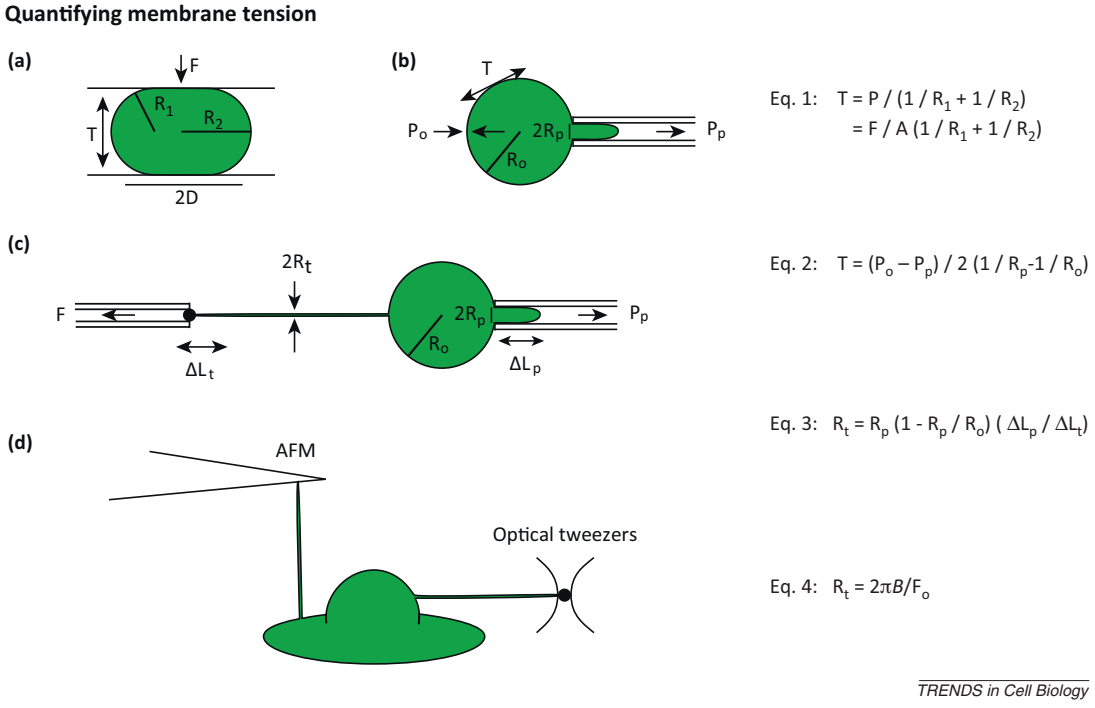

Figure I. Quantifying membrane tension. **(a)** The spherical cell or lipid vesicle is compressed with known force (F) between two parallel plates. R1 and R2 are the radii of the principal curvatures of the surface. The internal pressure P (P = F/A) is the applied force F divided by the contacted area ($A = \pi D^2$) between the plate and the cell or vesicle. This pressure is in equilibrium with the surface tension T (see Equation [1] in figure). **(b)** The membrane of a spherical cell or lipid vesicle is deformed by a micropipette. Pp is the pressure in the pipette, and Po is the pressure in the reservoir. Ro and Rp are the radii of the cell or vesicle and the pipette. The resulting isotropic stress in the membrane is the surface tension T and is determined by Equation [2] in figure. **(c)** Initially, tethers were formed using a micropipette to hold samples with suction pressure. A bead in a second pipette was used to extract a membrane tether with force F. Rp and Ro are the radii of the pipette and the cell or vesicle. The tether radius Rt can be calculated from the change in the length of membrane projection in the pipette (ΔLp) caused by the tether length change (ΔLt) as seen in Equation [3] in figure or can be derived from the membrane bending stiffness/static tether force relationship as seen in Equation [4] in figure [17] for cells with simple morphologies, such as red blood cells or lipid vesicles. **(d)** Atomic force microscopy cantilevers and optical tweezers provide higher-resolution measurements of plasma membrane (PM) tension [65,66,79,80]. See Box 2 for an in-depth description of how tethers can be used to measure membrane tension.

assembly [21]. Clathrin-mediated endocytosis is independent of actin dynamics in many circumstances but requires actin polymerization in others. On the apical surface of polarized cells where MCA is higher [21,29] or following cell swelling, actin engagement is necessary to convert a coated pit into a vesicle [21].

Caveolae

Caveolae, invaginations of the PM that are formed by caveolins, are physiological membrane reservoirs that have recently been shown to enable cells to accommodate sudden changes in PM tension [30,31]. Increases in tension through cell stretching or hypo-osmotic shock induce

Box 2. Quantifying membrane tension and MCAs from tethers

The mathematical relationship between tether force (F_o) and tension is known for lipid vesicles [15,63,71]. In cells, the tether force is generated by a combination of: (i) T_m of the lipid-bilayer; (ii) membrane bending stiffness (B); and (iii) MCA (γ) [17,72]. The T_m and cytoskeleton adhesion terms are difficult to separate and are therefore combined into a single term (T) that is known by multiple names: PM tension, apparent membrane tension, or effective membrane tension [17]:

$$T = T_m + \gamma = F_o^2/8B\pi^2 \quad [I]$$

(i) T_m is the result of the membrane being an inelastic fluid that equilibrates stresses within milliseconds [40,73]:

$$T_m = k(\Delta A/A) \quad [II]$$

where k is the elastic area stretch modulus, which depends on lipid composition, and A is the cell surface area.

T_m and k completely characterize the differential equation of state for planar surfaces. However, when the surface is not a plane (e.g., when it is rippled due to thermally driven fluctuations), measurements of these parameters include the entropic elasticity of the membrane.

T_m appears to be uniform throughout the whole cell, even across the junctions of epithelial cells [28], and on cell blebs in which the membrane separates from the actomyosin cortex [29,74].

(ii) The membrane bending stiffness (B) relates to the force needed to bend the membrane for a given radius of curvature. It has been experimentally measured for tethers in lipid vesicles. It is approximately 10^{-19} N m for a typical lipid bilayer, red cell, or neutrophil membrane [17–19]. It can also be calculated from measurements of tether radius as a function of the static force:

$$B = F_o R_t/2\pi \quad [III]$$

(iii) The MCA force can be expressed as adhesion energy per unit area (γ). It results from various MCA proteins that connect the actin cytoskeleton and the plasma membrane [75] and nonspecific binding of the membrane-to-cortex components. It was long believed that MCA was the result of only specific protein–protein interactions, but some experimental findings suggest otherwise. For instance, the fact that tether forces are rapidly reversible with no hysteresis has been used to favor a continuum model with nonspecific binding of the membrane-to-cortex components [17,24].

To separate MCA and T_m, we can measure F_b, the tether force in the absence of MCA contribution. This can be achieved experimentally by performing tether measurements on nascent blebs, which are locally devoid of cytoskeletal support, or on cells in which the cytoskeleton has been depolymerized. Under these conditions, the adhesion term (γ) equals 0 [29], and T_m is given by:

$$T_m = F_b^2/8B\pi^2 \quad [IV]$$

The adhesion energy can then be calculated using Equation [I] and [IV] if we assume that T_m is constant over smooth regions of the cell surface:

$$\gamma = T - T_m = (F_o^2/8B\pi^2) - (F_b^2/8B\pi^2) = (F_o^2 - F_b^2)/8B\pi^2 \quad [V]$$

A recent model has related pulling force–velocity profiles to the density of crosslinkers and the lipid bilayer viscosity [76], providing a possible means of discriminating the two PM tension components in a wider range of cellular contexts.

Experimentally, the tether force in cytoskeletally unsupported regions is typically less than half of that in regions supported by the cytoskeleton ($F_b < 0.5F_o$). Applying this inequality to Equation [V], we see that over 75% of the PM tension term is the result of MCA. T_m can increase markedly with hypotonic swelling [30,77]. However, under normal conditions, large changes in PM tension are thought to primarily reflect changes in MCA [78].

disassembly of caveolae, whereas recovery of iso-osmolarity leads to complete caveolar reassembly [30]. How caveolae buffer PM tension is not yet fully understood, because the amount of area released on membrane tension surge is very small (approximately 0.3%) [30].

Actin network assembly

To generate lamellipodium-like protrusions during cell crawling, growing actin filaments must generate sufficient local force to displace the PM [5,20,23]. Indeed, actin-based protrusion can lead to an increase in T_m as the force of polymerization unfolds wrinkles in the membrane during cell spreading [20]. Moreover, an increase in PM tension constrains the spread of the existing leading edge and prevents the formation of secondary fronts in chemotactic cells such as neutrophils [6]. In these cells, increasing cell tension by micropipette aspiration is sufficient to act as a long-range inhibitor of the signals that promote actin assembly at the leading edge. Conversely, the reduction of PM tension through hyperosmotic shock produces global activation of leading edge signals [6]. Because the front is the likely source of tension, any fluctuation in front size is immediately balanced by compensatory changes in tension levels, providing a possible mechanism of homeostasis [5,6].

Models of the PM as a global mechanical regulator

Several models suggest a role of PM tension as a global mechanical regulator that coordinates cell protrusion and retraction. PM tension has been suggested to optimize motility by streamlining filament polymerization in the direction of movement [22]. A model of actin network polymerization in an inextensible membrane bag can quantitatively predict both cell shape and speed and recapitulate the natural phenotypic variability in a large population of motile epithelial fish keratocytes [23]. If PM tension is assumed to be spatially homogeneous at all points along the cell boundary, the force per filament is inversely proportional to the local filament density. Therefore, at the center of the leading edge, the membrane resistance per filament is small, allowing filaments to grow rapidly and generate protrusion. As filament density gradually decreases towards the cell sides and the cell rear, the forces per filament caused by PM tension increase until polymerization is stalled and the actin network disassembles [23]. More recently, Ofer *et al.* [32] hypothesized a simple disassembly clock mechanism in which the rear position of a lamellipodium is determined by where the actin network has disassembled enough for membrane tension to crush the actin network and haul it forward. Finally, PM tension could also limit bleb expansion [33], but direct experimental evidence is still missing.

Role of cytoskeletal tension versus PM tension

Both cytoskeletal tension (also referred to as contractility) and membrane tension are capable of transmitting forces

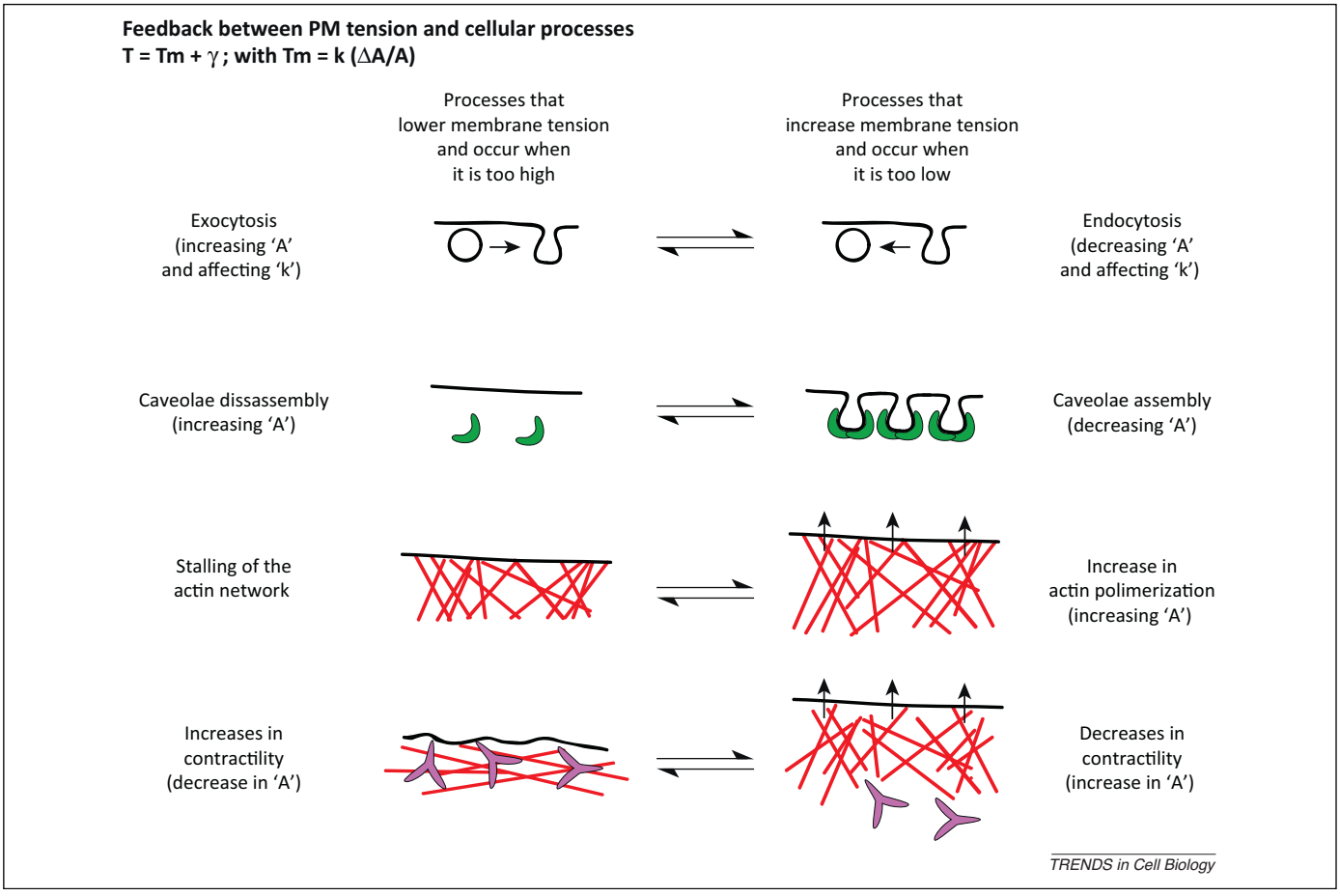

Figure 1. Feedback between plasma membrane (PM) tension and cellular processes. Examples of cellular processes that occur when PM tension is too high and that lead to its reduction (left) or that occur when PM tension is too low and lead to its increase (right) – vesicle trafficking, caveola formation, actin polymerization, and changes in myosin. In brackets we comment on the parameters of Equations [I] and [II] in Box 2 that are predicted to change in each of these processes.

over long range to spatially and temporally regulate cell polarity and cell migration [23,29,34]. Membrane tension antagonizes actin-based protrusion by being the barrier that growing actin filaments fight to protrude the membrane [23] and contractility opposes protrusion by pulling actin filaments away from the membrane [35]. The relative contribution of cytoskeletal versus membrane tension is likely to vary in different cell types.

In *Dictyostelium*, contractility plays an important role in restricting signals to the leading edge. Upon deletion of myosin 2, cytoskeletal tension is reduced dramatically [36] and there is an increase in lateral pseudopod number [37] and in Ras activation [38]. These data support a predominant role of contractility in *Dictyostelium* polarity, but whether PM tension also plays a significant role remains unknown.

In fibroblasts, a combination of cytoskeletal and membrane tension limits cell protrusion. Increasing membrane tension by hypo-osmotic shock halts spreading, whereas decreasing it by adding lipids increases the rate of cell spreading, enhances lamellipodial extension, and transiently causes uniform spreading [5]. Decreasing contractility through myosin inhibition causes faster spreading and a larger final spread area [35], and increasing it with biaxial cellular stretching downregulates Rac activity [39].

In fish keratocytes, decreasing contractility through myosin inhibition does not destroy keratocyte polarity and only slightly reduces migration speed, suggesting a predominant role for PM tension in this system [23,40].

In neutrophils, membrane tension also appears to be the dominant inhibitory mechanism for cell polarization. Membrane tension increases during neutrophil protrusion and decreasing membrane tension through hypo-osmotic shock results in the expansion of leading-edge signals and loss of polarity [6]. Decreasing cytoskeletal tension with myosin inhibition has no effect on leading-edge signals [6].

To what extent myosin inhibition, osmotic shock, or other tension perturbations affect both membrane tension and contractility remains unknown. Moreover, it is likely that cytoskeletal and membrane tension are interdependent; myosin 2 activity is required to reduce PM tension at the end of spreading [20], and its inhibition increases PM tension in resting neutrophils [6].

Finally, it is important to note that these conclusions (along with most other investigations of cytoskeletal tension) rely on myosin inhibition, but it is also possible that filament disassembly-based changes in cytoskeletal tension could contribute to cell polarity and movement in the absence of myosin activity.

Sensing PM tension

The molecular mechanisms by which cells sense and respond to mechanical signals are not fully understood.

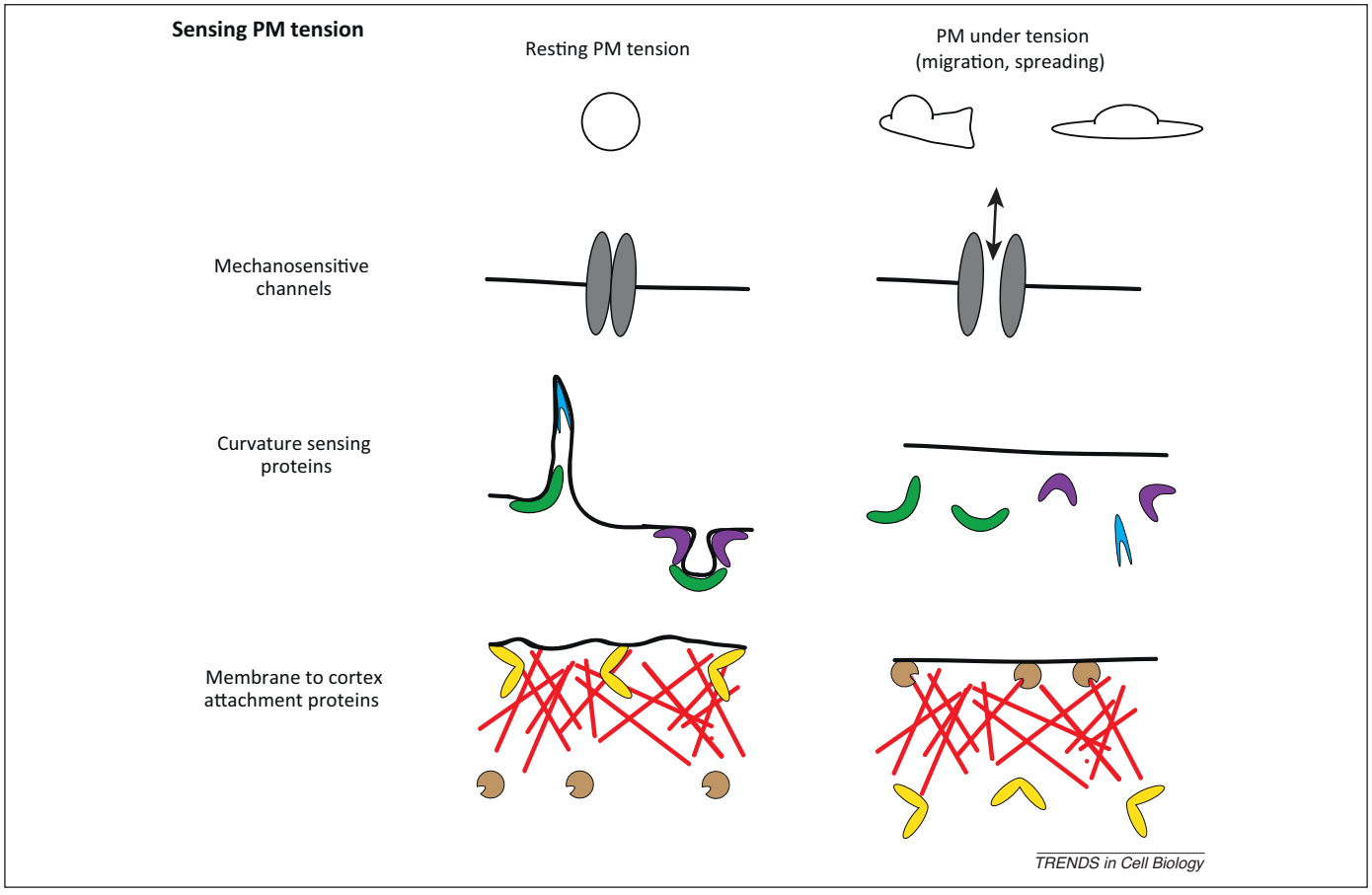

Figure 2. Sensing membrane tension. Plasma membrane (PM) in a resting cell (left) or following an increase in PM tension, as observed during cell protrusion or cell spreading (right). PM tension could be sensed by the opening of stretch-activated ion channels (top), the dissociation of curvature-sensitive membrane-binding proteins (middle), or changes in the activity of membrane-to-cortex attachment (MCA) proteins (bottom).

There are several mechanisms by which a cell could read PM tension (Figure 2).

Mechanosensitive channels

Stretch-activated ion channels are the best understood sensors of PM tension. For these channels, changes in PM tension affect the probability of channel opening. Some examples are found in prokaryotes (the ion channel MscL) [41], primary osteoblasts [42], and specialized sensory cells [43]. Mechanosensitive channels can sense membrane tension over a wide dynamic range. The magnitude of tension sensing varies from signals barely above the thermal noise in hair cells [43] to a set point for activation near the lytic tension of the bilayer for MscL [41].

Perturbation of the ion gradients across the PM and the influx/efflux of water can also dramatically increase/decrease PM tension. Osmotic changes have been used to manipulate PM tension [6,20,21,30], but whether cells use this mechanism to change membrane tension remains to be seen.

Curvature-sensing proteins

Numerous proteins have domains (like BAR or ALPS domains) that associate with curved membranes, either because they are sensitive to curvature or because they induce curvature (or both) (reviewed in [44]). High PM tension could reduce the binding of I-BAR proteins by limiting the membrane bending that is necessary for their binding to the membrane [45]. In this manner, the many GEFs and GAPs with curvature-sensing domains could regulate GTPases in a tension-dependent manner [46]. Indeed, ArfGAP1, which contains an ALPS domain, has a preference for positively curved membranes (like those generated during vesicle formation) or areas with disrupted packing of lipids. ArfGAP1 preferentially induces hydrolysis of the GTP of Arf in these regions [47,48]. High curvature could be both sensed and generated at the leading edge through the action of individual proteins such as amphiphysin I (BAR domain-containing effector in clathrin-mediated endocytosis), whose density on the membrane determines whether it senses or induces curvature [49]. Additionally, the activation of N-WASP-mediated actin polymerization by proteins containing an F-BAR domain depends on membrane curvature [50]. This suggests the possibility of feedback between curvature and actin dynamics [51]: curvature-sensing/inducing proteins could stimulate actin polymerization in a curvature-dependent manner, and actin polymerization could decrease curvature to maintain homeostasis.

MCA proteins

MCA proteins, which provide links between the PM and the actin cytoskeleton, could also sense PM tension. External forces have been found to modulate the activity

of some MCA proteins. Candidates include filamin [52] and the myosin 1 family of single-headed and membrane-associated myosins [53], both of which can interact simultaneously with the cytoskeleton and the PM. Filamin A is a central mechanotransduction element of the cytoskeleton that interacts with FilGAP, a GTPase-activating protein specific for Rac; the loss of this interaction due to high stresses increases Rac activation and actin polymerization [54]. Myosin 1c is an MCA protein that dynamically provides tension to sensitize mechanosensitive ion channels responsible for hearing [55]. Myosin 1b dramatically alters its motile properties in response to external force; the rate of myosin 1b detachment from actin decreases 75-fold under forces of 2 pN or less [56]). This suggests a potential mechanism that remains to be tested: if sensation of PM tension decreases MCA protein activity, it could also generate a homeostatic feedback loop.

Global versus local membrane tension

Asymmetries in contractility are sufficient to polarize both protrusion and adhesion. [57]. Are there also inhomogeneities in membrane tension, and if so are they functionally relevant? Membrane lipids flow like a liquid and can almost instantaneously equilibrate T_m across the cell [40]. The lack of large-scale flows has been interpreted as indicating uniform PM tension in several cell lines, including keratocytes [23]. However, lack of flow could also be achieved by the presence of local barriers that limit lipid movement, which are known to exist, at least over short timescales [58]. Interestingly, PM tension is inhomogeneous in epithelia and neurons [21,24,30,31] (reviewed in [4]). For these cells, it was observed that T_m is homogeneous across a cell, and only the MCA component differs between different membrane compartments. Is this always the case? A septin ring has been observed in T lymphocyte migration [59]; if the ring provides a lipid diffusion barrier, it could enable transient differences in PM tension in immune cells during movement. MCA and T_m can be distinguished by different means (Box 1), and future studies should assess the sources and prevalence of PM tension inhomogeneity. It is important to note that even uniform membrane tension could orchestrate the initiation and maintenance of cell polarity if it opposes cytoskeletal protrusions that are locally regulated (in which protrusions grow until they generate enough tension that enables some protrusions to survive and all others to be extinguished).

Future directions

There are many open questions regarding membrane tension. Do cells have a set point for PM tension? If so, how is it maintained? Does membrane tension regulation differ for isolated cells versus cells in a tissue? How do motile cells interpret changes in PM tension? How do cytoskeletal tension and PM tension interrelate? Further research will be necessary to determine which signaling currencies are altered by changes in PM tension and to clarify how membrane tension contributes to and is affected by endocytosis, exocytosis, actin dynamics, and myosin activity.

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