Topical Review

Membrane tension and cytoskeleton organization in cell motility

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Abstract
Cell membrane shape changes are important for many aspects of normal biological function, such as tissue development, wound healing and cell division and motility. Various disease states are associated with deregulation of how cells move and change shape, including notably tumor initiation and cancer cell metastasis. Cell motility is powered, in large part, by the controlled assembly and disassembly of the actin cytoskeleton. Much of this dynamic happens in close proximity to the plasma membrane due to the fact that actin assembly factors are membrane-bound, and thus actin filaments are generally oriented such that their growth occurs against or near the membrane. For a long time, the membrane was viewed as a relatively passive scaffold for signaling. However, results from the last five years show that this is not the whole picture, and that the dynamics of the actin cytoskeleton are intimately linked to the mechanics of the cell membrane. In this review, we summarize recent findings concerning the role of plasma membrane mechanics in cell cytoskeleton dynamics and architecture, showing that the cell membrane is not just an envelope or a barrier for actin assembly, but is a master regulator controlling cytoskeleton dynamics and cell polarity.

Keywords: membrane tension, cell motility, cytoskeleton

1. Introduction to membrane/cytoskeleton interactions in different motility modes

Animal cells, as opposed to plant cells, have no cell wall, so the plasma membrane is what holds the cellular contents together, contributes to shape and size determination, and enables the cell to interact with its environment. The cell membrane also serves as the physical scaffold where actin cytoskeleton assembly is initiated via the recruitment and activation of actin polymerization nucleation and filament elongation factors [1]. The growth of a branched actin network beneath the plasma membrane allows the protrusion of the membrane when cells are adherent and crawling on a substrate, known as gliding cell motility [2]. The cellular protrusion in this case is known as a lamellipodium, composed of a branched array of actin filaments, which is often interspersed with other more specialized protrusive fingers called filopodia, composed of aligned, unbranched filaments (figure 1). Another type of membrane-associated actin structure is the cellular cortex, a network of both branched and unbranched actin filaments and myosin, which closely underlies the cell membrane and is attached to it [3, 4]. This is a contractile structure, owing to tension produced by myosin motor activity in the network, with higher myosin activity being associated with higher cortical tension (for review [5]). The cortex is important for pulling up and squeezing forward the back of the crawling cell and also for the formation of the other main cellular protrusion, the bleb (figure 1). Although long considered the hallmark of a dying cell, membrane blebs have gained recognition in the past few years as important cellular protrusions involved not only in lamellipodia-independent motility but also in cell shape homeostasis [6, 7]. Blebs form when either the acto-myosin cortex itself or the connection between the cortex and the plasma membrane is disrupted, producing a patch of cell...
Figure 1. Probing the cell membrane. Representation of a moving cell, showing the acto-myosin organization in the most important structures in a moving cell: cell cortex, blebs, lamellipodia and filopodia. The main techniques for probing cell membrane and cortex properties are shown: (a) micropipette aspiration, with an inset concerning the Laplace law used to analyze the experiment, (b) tether pulling, with the equation relating the tension of the bilayer ($\sigma$) to its bending stiffness ($\kappa$) and the tether force ($f$) as measured by an optical trap and (c) interferometry, which measures spontaneous fluctuations of the cell membrane with high spatio-temporal resolution using an optical trap. Zooms on the cortex and on the base of the tether show the actin network interspersed with myosin minifilaments and bound to the membrane via links such as ezrin.

membrane that is no longer connected to the actin cytoskeleton (figure 1) [8,9]. The free membrane expands outward, and subsequently the acto-myosin cytoskeleton reforms at the bleb membrane. The protrusion thus produced can be used by the cell to pull itself forward, particularly in confined environments [10]. Lamellipodia (polymerization-based) and blebs (contraction-based) can co-exist, or combine to give hybrid modes such as the lobopodia [11].

The close association of the actin cytoskeleton and the cell membrane means that the membrane could affect the cytoskeleton for purely mechanical reasons, unrelated to the role of the membrane in biochemical signaling cascades. There have been several excellent reviews concerning this subject[12–14], and our goal here is to provide the latest update on evolutions in the field over the past few years.

2. Introduction to membrane mechanics

2.1. Energy of membrane deformation

One of the main mechanical characteristics of a membrane is its bending stiffness $\kappa$. The bending stiffness resists the generation of local membrane curvature, and is a constitutive parameter that depends on the local composition of the membrane. The membrane bending energy (per unit area) can be described by the following expression, dependent on the bending stiffness and the square of the local membrane curvature ($C$): $E_{\text{bend}} = (\kappa/2)C^2$. Membrane bending stiffness is generally a few times the thermal energy, around 20 $k_B T$ with $k_B T \approx 4 \times 10^{-21}$ J or 4 pN nm at 24 $^\circ$C, as discussed in section 2.3.2. Moderate membrane bending therefore occurs
spontaneously at room temperature due to thermal fluctuations, but the creation of thin tubular extensions, associated with a large change in membrane curvature, requires the generation of active stresses.

Another main mechanical characteristic of a membrane is its tension $\sigma$, henceforth called the in-plane tension. The in-plane tension of the membrane resists an increase in membrane area, and is a measurement of how taut the membrane is. Anything that increases the apparent surface area of the lipid bilayer, for example cell adhesion to the substrate or hypo-osmotic shock treatment, will increase membrane tension, while de-adhesion or hyper-osmotic shock will lower tension. Indeed, a membrane with an apparent area of $A_0$, as seen for instance by an optical microscope, has in reality a larger (true) area, which is proportional to the number of lipids. In liposomes, since the number of lipids does not change during membrane stretching, the tension ratio between a vesicle having an apparent area $A_0$ and the same vesicle stretched to an apparent area $A_0 + \Delta A$ is of entropic origin, and comes from the reduction of membrane fluctuations upon stretching. It is therefore a function of the membrane bending rigidity and the temperature according to: $\sigma / \sigma_0 = e^{-\kappa A / (2k_BT)}$ where $\kappa$ is the membrane bending stiffness discussed above [15]. This expression cannot be directly used for cells, because the cell membrane is not at thermal equilibrium and is subjected, amongst other things, to cytoskeletal forces. Furthermore the number of lipids can change due to membrane exchange processes like exo and endocytosis. Nevertheless, this expression illustrates that variations of the apparent area of a cell are likely correlated with variations of its membrane tension on short time-scales, before cellular processes of active tension adjustment come into play.

The interplay between membrane bending rigidity and membrane tension in defining membrane shape and energy can be seen by studying thin membrane tubules such as those formed by the local application of an external force pulling on the membrane. Neglecting membrane deformation at both ends of the tubule and assuming that it is a perfect cylinder of radius $r_t$ and length $L_t$, the deformation energy of the tubular membrane takes into account the cost of bending the membrane and working against membrane tension: $E_t = (\pi L_t \kappa / r_t^2) + 2\pi r_t L_t \sigma$. If the tubule is empty, its optimal radius is when the tube energy is minimized: $(\partial E_t / \partial r_t) = 0 = - (\pi L_t \kappa / r_t^2) + 2\pi L_t \sigma$, leading to $r_t = \sqrt{\kappa / 2\sigma}$. Inserting the optimal radius back into the tube energy, and differentiating with respect to the tubule length gives the force: $f_t = (\partial E_t / \partial L_t) = \pi \sqrt{2\pi \sigma} + \pi \sqrt{2\pi \sigma} = 2\pi \sqrt{2\pi \sigma}$. This expression illustrates that the force necessary to create a thin membrane tubule depends on both the membrane tension and its stiffness.

However with $\kappa = 20$ $k_BT$ and an average cellular membrane tension value of about 100 pN $\mu$m$^{-1}$ (see section 2.3.2), $r_t$ is small, about 20 nm. Cellular protrusions such as filopodia are filled with actin, and the membrane radius of curvature is typically much larger than $r_t$, meaning that the cost of curving the membrane is less important. For larger cellular structures such as lamellipodia, the curvature does not change much as the cell crawls forward, and therefore membrane curvature is likewise not important for leading edge dynamics. However actin assembling beneath the membrane to push it forward in both kinds of cellular structures will still have to work against the membrane tension. It is therefore key to measure the membrane tension in order to understand lamellipodia and filopodia dynamics.

### 2.2. Cell tension is a combination of membrane tension and cortical tension

The caveat is that the membrane often interacts tightly with the actin cytoskeleton, and thus distinguishing the contribution of each component to the overall tension of the cellular interface is not straightforward. The experimental difficulty of probing just the membrane has sometimes lead to a confusion between the ideas of membrane tension, cortical tension and cell tension, terms that specify different parameters but are often mistakenly used interchangeably. In-plane membrane tension reflects only the properties of the lipid bilayer, independent of how the bilayer interacts with the underlying cytoskeleton. Cortical tension on the other hand is a measure only of the isolated acto-myosin network, without the membrane. Cell tension is the combination of membrane tension and cortical tension, and is the most experimentally accessible parameter.

To understand why membrane and cortical tensions add up to counteract large-scale cellular deformation ($\sigma_{\text{cell}} = \sigma_{\text{mem}} + \sigma_{\text{cor}}$, zoom on the cortex in figure 1), we must consider that the cortex and the membrane are connected by molecular linkers. These linkers are put under tension when the membrane moves away from the cortex. The force per unit area of the cortex-membrane connection is proportional to the force per linker times the linker density $f_{\text{link}} \rho_{\text{link}}$. The pressure difference $\Delta P$ between the exterior and the interior of the cell acts essentially on the membrane since the cortex is a permeable structure that can sustain little osmotic or hydrostatic pressure. Therefore, a force balance (per unit area) on the membrane implies $\Delta P = \sigma_{\text{mem}} C + f_{\text{link}} \rho_{\text{link}}$, where $C$ is the local curvature. However the linkers are themselves put under tension by the cortical tension $\sigma_{\text{cor}}$ created by acto-myosin contraction, implying $f_{\text{link}} \rho_{\text{link}} = \sigma_{\text{cor}} C$. Combining these two relations leads to the generalized Laplace law $\Delta P = (\sigma_{\text{mem}} + \sigma_{\text{cor}}) C$, which shows that the membrane and cortical tension contribute additively to the overall cell tension [16]. This relation explains why it has been observed that membrane tension in resting neutrophils increases upon myosin inhibition [17]. All other things being equal, a decrease in $\sigma_{\text{cor}}$ in the preceding equation means that $\sigma_{\text{mem}}$ must increase in compensation. One can imagine that when the cellular contents are no longer compressed by the active acto-myosin cortex, they expand, flattening out membrane folds and increasing tension.

### 2.3. Experimental techniques for probing the cell membrane and measuring membrane tension

#### 2.3.1. Micropipette aspiration

One way of probing membrane properties is by micropipette aspiration (figure 1 and [18]). For this the surface of a liposome or cell is sucked into a $\sim 5$ $\mu$m-diameter micropipette to make a hemispheric protrusion, and the tension is calculated, knowing the amount
of pressure needed to aspirate the cell and by making use of Laplace’s law (inset, figure 1). This technique has been successfully used to measure the in-plane membrane tension of giant liposomes, provided that the aspirated portion remains small. Beyond this point, pulling more area into the pipette changes the membrane tension by pulling out surface undulations and increasing the membrane projected area [15]. Keeping this in mind, micropipette aspiration can be applied to cells in suspension, but is more problematic for adherent cells due to the fact that the part of the cell outside of the pipette is non-spherical. This can be circumvented if detailed geometry of the adherent cell is available [19], but to apply this approach, the cell has to be relatively uniform in shape, something that is rare in most motile cells. A solution is to de-adhere cells and allow them to become rounded and then perform micropipette aspiration. However this undoubtedly changes the physiology of the cell and thus perhaps the tension. For example de-adhesion is known to give an overestimation of tension in fibroblasts because these cells decrease their tension upon spreading [19, 20]. In all cases, micropipette aspiration gives access to the overall tension of the cell interface, which is a combination of the membrane tension and the tension in the underlying acto-myosin cortex. Indeed perturbing both myosin I and II motor function in various cell types has been shown to strongly affect tension as measured by micropipette aspiration [21, 22]. One micropipette study of fibroblasts growing in suspension indicated that membrane tension is negligible compared to cortical tension: 413.6 pN μm\(^{-1}\) for an untreated cell versus 39.1 pN μm\(^{-1}\) for a cell whose actin cytoskeleton had been disassembled by cytochalasin D [22]. So for all intents and purposes, aspiration in this case gives direct access to cortical tension values. This may not be the case for all cell types and drug treatments could change the tension in indirect ways, so for this reason, micropipette aspiration is a technique that is more appropriate to measuring the overall cellular tension rather than the membrane tension itself.

2.3.2. Pulling membrane tethers Another way to measure membrane tension is by pulling membrane tethers from the plasma membrane using laser tweezers [23, 24]. As illustrated in figure 1, this technique involves attaching a bead to the cell membrane and pulling a membrane tube with a laser tweezers to measure how much force is needed to maintain the tube. As discussed above, for a simple membrane, this force is determined by the tension of the bilayer (σ) and its bending stiffness (κ), and the membrane tension can be related to the tether force (f) by the following expression: \( σ = f^2 / 8\pi^2κ \) [25]. The bending stiffness can be determined without knowing the membrane tension provided both the tether force and the tether radius \( r_t \) can be measured: \( κ = fr_t / 2\pi \). Although tether radii are typically very small and below optical resolution, they have been measured by careful densiometry analysis of either differential contrast or fluorescent images [26, 27]. Bending rigidities of biological lipidic membranes thus calculated tend to be on the order of 10\(^{-19}\) N m, varying from keratocytes 1.4 \( \times \) 10\(^{-19}\) N m [27] to neuronal growth cones 2.7 \( \times \) 10\(^{-19}\) N m [26]. See also [26] for a list of measured values in liposome systems, which give roughly identical values. Since bending rigidities vary little for biomembranes, most tether pulling studies of membrane tension use a value of about 20 \( k_B T \) for κ without measuring it for each cell type. To give an idea of approximate values, cellular membrane tensions tend to be on the order of 100 pN μm\(^{-1}\), so in order to pull membrane tethers, forces in the range of 25 pN must be applied.

In liposomes, the bending stiffness is set by the membrane composition, and the membrane tension depends on the external conditions, such as osmolarity and adhesion to the substrate. The tether force is therefore typically well-defined under given conditions, independent of the tether length, and only weakly affected by the speed of extraction, with a fast relaxation to its static value. The situation is quite different once other components, for example membrane proteins, are incorporated into a liposome. In this case, the tether force depends strongly on the speed of extraction and displays slow relaxation (up to several hundreds of seconds) to its static value. This difference can be traced to an increased friction force due to impurities, and/or to the slow diffusion of impurities out of the membrane tube [28]. The identity of the bilayer contaminant is not important, the real danger being the comparison between ‘clean’ liposomes and those with added components. In cells where membranes are never pure, these dynamic effects need to be taken into consideration, and tethers need to be pulled slowly and allowed time to relax.

Although the tether is devoid of cytoskeleton and the tether force reflects the actual membrane tension in the tether, there is a major complicating factor with relating this value to the cell membrane tension. As depicted in figure 1, pulling a membrane tube from the surface of a cell involves working against in-plane tension, but also the connection between the membrane and the underlying cytoskeleton. The proportion of a measured tether force contributed by real in-plane membrane tension versus cytoskeleton attachment is something that must be empirically determined for each cell type. This can be evaluated by pulling tethers from several different regions of the cell that have different cytoskeleton organizations, for example the front edge versus the back edge of a cell or on blebs, that contain no cytoskeleton at all in the early phases of their growth. If tether force values are identical, this is a good indication that the extraction is really probing in-plane membrane tension, with little contribution from the cytoskeleton. Some cell types, like Caenorhabditis elegans sperm cells or mitotic HeLa cells, appear to have little or no contribution of cytoskeleton to measured tether values [29, 30], while tether forces on other cell types such as renal epithelial cells and melanoma cells seem to be almost only a measure of cytoskeleton attachment [25]. Table 1 is a non-exhaustive summary of measured tether forces and, where appropriate, in-plane membrane tension values showing that membrane tensions cover a wide range, from 3 to 276 pN μm\(^{-1}\). We will discuss later how active cellular processes contribute to membrane tension homeostasis, keeping in mind that these values are far from the rupture value for a cell, 3000–10 000 pN μm\(^{-1}\) [31, 32] and references therein.
Table 1. Tether forces and calculated membrane tension values for various cell types under different conditions. Values obtained on blebs or upon cytoskeleton disruption, as well as those entries marked **, are real in-plane membrane tensions, while the others are apparent membrane tensions, possibly including a cytoskeleton attachment component.

<table>
<thead>
<tr>
<th>Tether force (pN)</th>
<th>Membrane tension (pN/µm−1)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. elegans sperm cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—isotonic conditions**</td>
<td>35</td>
<td>150</td>
</tr>
<tr>
<td>—hyperosmotic shock**</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Keratocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—no treatment</td>
<td>54</td>
<td>276</td>
</tr>
<tr>
<td>~40</td>
<td>Not calculated</td>
<td>[36]</td>
</tr>
<tr>
<td>—on blebs</td>
<td>~33</td>
<td>~100</td>
</tr>
<tr>
<td>—actin cytoskeleton disruption (cytochalasin)</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Melanoma cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—on blebs</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>—on attached membranes</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>—actin cytoskeleton disruption (cytochalasin)</td>
<td>Not applicable</td>
<td>18</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—on blebs</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>—on apical membranes</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—resting</td>
<td>8.5</td>
<td>Not calculated</td>
</tr>
<tr>
<td>—activated (chemoattractant addition)</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>—inhibit myosin</td>
<td>~14</td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells, epithelial-like cells and brain tumor cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—All three cell types, actin cytoskeleton disruption (latrunculin)</td>
<td>~15</td>
<td></td>
</tr>
<tr>
<td>Mitotic HeLa cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>—on glass**</td>
<td>~20</td>
<td>Not calculated</td>
</tr>
<tr>
<td>—on fibronectin**</td>
<td>~30</td>
<td></td>
</tr>
</tbody>
</table>

*κ used to calculate the membrane tension from the tether force ranged from 1–3 × 10−19 N m.

**Tubes pulled in different regions of the cell with different cytoskeleton organizations give identical values, so contribution of cytoskeleton attachment to tether force is considered negligible.

2.3.3. Interferometry. A newly developed alternative method to tube pulling and micropipette aspiration for measuring in-plane membrane tension uses a non-invasive high-speed interferometric approach to detect membrane fluctuations ([33, 34] and figure 1). Fluctuations can be related to the membrane tension, but as with membrane tethers and micropipette aspiration, if the cytoskeleton is attached to the membrane, the fluctuations also reflect the acto-myosin dynamics of the cell. When cytoskeleton is destroyed however, this technique gives comparable values to those obtained by tube pulling on cell blebs [35] (table 1), and thus is a promising complement to existing membrane tension measurement techniques, with the advantage that unlike tether pulling and micropipette aspiration, the cell is not physically manipulated during the measurement.

3. What sets the in-plane membrane tension of a cell and how is it maintained?

There is a large dispersion in the membrane tension values measured for different cell types, even when comparing only those values that represent pure membrane tension (table 1). This observation begs the question as to what sets the membrane tension of different cell-types.

3.1. Role of membrane trafficking in tension homeostasis

During spreading of fibroblasts, membrane area is observed to increase by 80% with the doubling of the cell area in contact with the substrate, and other cell types show similar trends although less dramatic [39]. Membrane tensions of unspread and spread cells as measured by tether pulling are identical. Without addition of membrane, a lipid bilayer will rupture upon a 3–5% increase in area, due to excessive membrane tension [31]. Something therefore must compensate for the large changes in cell and membrane area required for a cell to spread once membrane folds have been pulled out. One membrane resource available to the cell is caveolae, membrane invaginations that have a stable and defined signature, unlike membrane folds and thermal fluctuations. When cells experience an increase in membrane tension, triggered either chemically or mechanically by physically increasing cell area, caveolae are predicted theoretically and observed experimentally to disassemble [40–42]. This is calculated to give approximately a 0.3% increase in membrane tension.
area per cell [41]. This number may appear small in regards to the large variation of cell volume observed during hypo-osmotic shock (halving the osmolarity), but it should be remembered that tension increase is a very non-linear function of area change (see section 2: \( \sigma / \sigma_0 = e^{(\Delta A / A_0)} \)). The rationale made in [41] was that most of the volume increase upon hypo-osmotic shock leads to the smoothing out of large-scale membrane folds, without a sizeable increase in membrane tension. It is only once thermal fluctuations start to be constrained that tension rises, so that the release of a mere 0.3% of membrane area contained in the ‘caveolae reservoir’ at this stage can have a large buffering effect on membrane tension. So caveolae may indeed act as a first responder to membrane tension increases by simply flattening out in response to stress.

However not all cell types possess large amounts of caveolae, and global cell shape change processes such as spreading require the mobilization of much more membrane than caveolae can provide. Another source of membrane tension buffering is exo- and endocytosis. In spreading fibroblasts, about half of the plasma membrane of the cell in its final spread state is estimated to come from exocytosis of a specific population of endomembranes, and waves of exocytosis are observed to follow transient spikes of membrane tension [39, 43]. Likewise induced decreases in membrane tension in mitotic HeLa cells produce increases in endocytosis [44]. Exo and endocytosis are active, selective processes, but in vitro experiments show that there could be a passive component in tension regulation as well: vesicles in proximity to an abruptly tensed lipid bilayer will fuse with the membrane, and when the membrane is relaxed, tubules will form to take up excess membrane [45]. Such events could likewise be occurring in cells.

### 3.2. Role of cytoskeleton assembly and cell-substrate adhesion in tension homeostasis

Despite these mechanisms to regulate tension, there does not seem to be a certain tension to which all cell types converge. In fact, although more data is needed, it seems that hypermotile cells such as the nematode sperm cell and the keratocyte display higher membrane tensions than more sedentary cells like epithelial cells (table 1). A possibility that has been shown for the keratocyte is that the actin cytoskeleton is one of the major determinants of membrane tension, and that within a certain window, extra membrane can be added to these cells without perturbing their movement and without changing their membrane tension [27]. Instead the actin cytoskeleton converts more of its pool of globular actin into filaments that fills in the extra membrane to make a larger protrusion, restoring the initial membrane tension. Actin assembly can thus regulate membrane tension, and since different cell types have different quantities of actin and actin binding proteins [46], this could lead to different resting membrane tensions for different cell types.

In this scenario, the characteristic membrane tension of a given cell type could be the tension at which actin polymerization at the cell leading edge is \( \Sigma_{\text{actin}} = f_{\text{actin}} \rho_{\text{actin}} \), where \( f_{\text{actin}} \) is the force per filament and \( \rho_{\text{actin}} \) is the filament density (per unit length). This force is directly acting on the leading edge membrane, and force balance requires \( \Sigma_{\text{actin}} = 2 \sigma \), where the factor 2 accounts for the dorsal and the ventral lamellipodium surfaces [27]. For the keratocyte, the stall force for one actin filament has been estimated as \( f_{\text{actin}} \approx 4 \) pN [47]. Using \( \rho_{\text{actin}} \approx 100 \) filaments \( \mu m^{-1} \), one finds \( \sigma_{\text{actin}} \approx 200 \) pN \( \mu m^{-1} \). Since the membrane tension of the keratocyte is similar to \( \sigma_{\text{actin}} \) (table 1), this suggests that the cytoskeleton assembles until membrane tension reaches a point where it is significantly contributing to slowing actin polymerization. Generalizing to other cell types, the \( \sigma_{\text{actin}} \) characteristic of the cell, as defined by the architecture of its cytoskeleton, would then define the homeostatic membrane tension maintained by the cell.

Another factor that could come into play in determining membrane tension is different adhesiveness amongst different cell types, with higher adhesion being associated with a higher membrane tension if the cell does not adjust its tension via the pathways discussed above. Keratocytes display a higher tension on more adhesive substrates as do erythrocytes in an artificially adhesive environment, and biomimetic liposomes also increase their membrane tension upon adhesion to the substrate [27, 48, 49].

#### 3.3. Is membrane tension uniform in a given cell?

Since the cell membrane is a fluid, gradients of membrane tension should lead to membrane flow from regions of low tension towards regions of high tension, giving a rapid equilibrium of membrane tension over the cell surface. Permanent gradients of membrane tension could be maintained out of equilibrium by processes such as targeted exocytosis at one location, leading to tension reduction, and targeted endocytosis at a different location, leading to tension increase. Permanent tension gradients could also be expected in moving cells due to frictional forces, as shown in two recent theoretical studies in moving cells, with the hypothesis that gradients could be maintained in the plasma membrane due to the friction caused by local obstacles, such as protein attached to the cytoskeleton and/or the substrate [50, 51]. A comparable situation is the hydrostatic pressure in the cell cytosol, which has been shown not to equilibrate instantly over distances as small as 10 \( \mu m \) [52]. Indeed, blebs can be suppressed on one side of a cell and not the other by local drug treatments that inhibit contractility. However the physics of how pressure gradients are maintained in a cellular cytoplasm are distinct from that which may be operational in the plasma membrane. Gradients of membrane tension could in principle be measured by pulling membrane tethers at the front, the side or the back of motile cells, with the caveat that it is very difficult with current methods to distinguish gradients in membrane tension from gradients in membrane-cytoskeleton attachment. Experimentally there is to date little evidence for gradients in membrane tension.
4. How membrane tension controls cytoskeleton dynamics and organization

In the previous sections, we have seen how cytoskeleton dynamics or processes dependent on cytoskeleton can alter membrane tension. In this section we examine the converse: how membrane tension can control actin cytoskeleton architecture and dynamics in *in vitro* systems, in blebbing cells and in polymerization-driven cell shape changes.

4.1. Membrane tension and cytoskeleton in *in vitro* systems

Confine ment is known to create spontaneous organization in polymer systems in general, including biopolymers. When microtubules above a threshold density are confined in a thin slit, they are found to undergo an isotropic-nematic type transition and self-align [53]. Likewise actin filament preparations in microchambers are sensitive to the size and aspect ratio of the chamber, and align with the walls or along the chamber diagonal, depending on conditions [54].

Similarly to these confinement effects, the presence of lipid bilayers has been shown to organize the actin cytoskeleton *in vitro*. For example bundled actin protrusions resembling filopodia spontaneously emerge from branched actin networks, resembling those in the lamellipodia of a moving cell, growing against a liposome membrane (figure 2(a) and [55]). This was shown to require a certain threshold membrane tension, and when the tension was reduced by osmotic shock, bundles did not form. An effective attractive force between two protrusions imposed by the plasma membrane and depending in part on its mechanical properties has also been predicted by modeling [56, 57]. Taken together, these studies show that purely mechanical effects of the tension of the plasma membrane on a growing actin network are sufficient to transform a lamellipodia-type branched network of the plasma membrane on a growing actin network into a filopodia-type bundled architecture, despite identical biochemical conditions. Other studies *in vitro* on non-deformable supported lipid bilayers show that filopodia can self-organize via sequential recruitment of different biochemical factors [58]. So it appears that there are purely mechanical or purely biochemical ways to produce a given architecture of an actin network *in vitro*, and it is probable that the cell uses a combination of these mechanisms. A good example of this comes from the bacteria *Listeria*, which move in the host cytosol by forming a branched actin comet at the bacterial surface. When these bacteria protrude against and deform the host cell plasma membrane, the filaments in the actin comet align and branching is reduced, at the same time that the biochemical profile of the comet is changed [59, 60].

4.2. Membrane tension and cytoskeleton in blebbing cells

At early time points in its life, a bleb is a naked bulge of membrane. This membrane is ballooned outward by the internal pressure of the cell, which derives from combined effects of osmotic pressure and cortical contractility (figure 2(b)). The energy of a bleb can be described as consisting of three parts: the work of the pressure difference $\Delta P$ to inflate a bleb of volume $V_b$, the cost of unbinding an area $S_b$ of membrane from the cortex, and the work against membrane tension during bleb inflation [61, 62]:

$$E_b = -\Delta P V_b + \epsilon S_0 + \sigma_{mem} \Delta S_b$$

where $\epsilon$ is the membrane-cortex adhesion energy and $\Delta S_b$ is the excess area contained in the bleb. Small blebs can be treated as spherical caps. Taking a uniform curvature $C_0$ with $S_0 C_0^2 \ll 1$, we can write:

$$V_b = \frac{S_0}{2} C_0 / 8 \pi$$

and $\Delta S_b = \frac{S_0^2 C_0^2}{16 \pi}$. Optimizing the bleb with respect to $C_0$ at fixed boundary area $S_0$ gives:

$$\frac{dE_b}{dC_0} = 0 = - (\Delta P S_0^2 / 8 \pi) + (\sigma_{mem} S_0^2 C_0 / 8 \pi)$$

and the Laplace law in the bleb:

$$C_0 = \Delta P / \sigma_{mem}$$

The Laplace law for the cell as a whole (section 2.2) is:

$$\Delta P = (\sigma_{mem} + \sigma_{cor} C_{cell})$$

where the cell curvature is $C_{cell} = \sqrt{16 \pi S_{cell}}$ for a spherical cell. Combining these two Laplace laws gives the bleb energy as:

$$E_b = \epsilon S_0 - (\sigma_{mem} + \sigma_{cor} S_{cell}) S_0^2$$

The bleb energy describes a nucleation process where if a patch of membrane, $S_0$, larger than a critical size detaches from the cortex, it is unstable and inflates to a full-grown bleb. This critical size is defined by $\Delta P = (\sigma_{mem} + \sigma_{cor} S_{cell}) S_0^2$. The bleb energy barrier to bleb formation is reduced by a critical size $S_{crit}$, presumably via myosin upregulation or weakening of cortex-membrane attachments, although how the mechanical constraint signals to these effects is unclear [64].

As to low membrane tension promoting blebbing, evidence is difficult to obtain since experimental treatments to change membrane tension, like osmotic shock, also change cellular internal pressure. For example *Dictyostelium* stop blebbing in hyperosmotic solution. Since hyperosmolarity deflates the cell and reduces membrane tension, one could expect this treatment to encourage blebbing, but the drop in membrane tension is associated with a lower internal pressure, thus reducing the driving force for bleb expansion [63]. However a mixed experimental/theoretical study showed that decreases in membrane tension are associated with increased bleb formation [22], and rapid de-adhesion experiments point in the same direction [62]. In the latter experiment, when cells are forced rapidly into suspension by digestion of their surface receptors, extensive blebbing behavior is observed, presumably due to a sudden decrease in membrane tension while cellular osmotic pressure and cytoskeleton remain unaffected [62]. Blebbing progressively decreases and ceases after some time (on the order of $\sim 5$ min) in an endocytotic-dependent fashion. On the other hand, blebbing persists for more than one hour when the endocytotic machinery is inhibited, and is not observed in wild-type cells when de-adhesion is performed more slowly, allowing endocytosis of extra membrane to keep pace with membrane release.
from adhesion sites. In a similar vein, other studies showed that cells undergoing mixed lamellipodial/blebbing motion spontaneously increase the frequency of lamellipodia formation upon migration into a more adhesive area on a patterned substrate [20]. Increased adhesion could increase membrane tension, making blebs less favorable and increasing the predominance of lamellipodial protrusions. In another study involving a mixed motility mode, blebs are observed to originate in concave regions, predominantly on the sides of existing lamellipodial protrusions or the sides of other blebs [65]. The physical argument is that in negatively curved regions, membrane tension helps the bleb form by exerting an outward directed force, instead of opposing bleb expansion as is the case in convex regions. However, the force from cortical tension is also outward in this geometry and should thus promote cortex-membrane cohesion, so the situation is unclear.

4.3. Membrane tension and cytoskeleton in polymerization-based cell shape changes

Polymerization-type modes of cell shape changes include not only whole cell translocation on a substrate, but also earlier phases of cell interaction with its substrate, namely spreading
and protrusion. In this type of motility mode, unlike the pressure-driven expansion of membrane that happens in a bleb, the membrane must be pushed out by cytoskeleton assembly. Assembly factors physically attach the actin network to the membrane. However the links between membrane-bound actin polymerization activators and the actin network are transient, so the membrane can detach to move forward and then reattach for subsequent rounds of polymerization. This mixed pushing/braking dynamic (a.k.a polymerization ratchet) is the basis of cell shape changes involving actin deformation of membranes. If the membrane is too far from the network, new material incorporated at growing filament ends is not felt by the membrane and it does not deform. On the other hand, if the membrane is too close to the growing actin network and is too tense to allow for the addition of new monomers to the growing filament tip, it may stall polymerization, as was already discussed in section 3.2. Polymerization stalls against the membrane if the actin network adheres tightly to the substrate, however in the case of lower adhesion, the actin filaments in the lamellipodium undergo retrograde flow, a backward movement away from the lamellipodium edge due to slippage between the cell cytoskeleton and its substrate adhesion complexes [66].

The protrusion velocity of a moving cell is then the difference between the actin polymerization velocity \( v_p \) and the retrograde flow velocity \( v_r \), meaning that in situations of high retrograde flow, polymerization power is wasted because the cytoskeleton network slides backwards when filaments assemble at the membrane instead of pushing it out. Although clear experimental evidence is lacking, the expectation is that membrane tension promotes retrograde flow [67–69]. Assuming that tension indeed regulates flow and that the traction force exerted by the cell is linear with its retrograde flow velocity, force balance at the lamellipodium edge reads \( \alpha v_r L_1 = 2\sigma_{mem} \). Here, \( \alpha \) is a friction parameter associated with actin sliding over the adhesion sites at a speed \( v_r \), and \( L_1 \) is the length over which the retrograde flow takes place, i.e., the length of the cellular protrusion. In addition to the ‘stall membrane tension’ described in section 3.2 that suppresses actin polymerization, there exists a second stall tension for which the cell movement stalls because the actin retrograde velocity matches the polymerization velocity, given by \( \sigma_{mem(stall)} = \alpha v_p L_1/2 \). Traction force measurements show that the lamellipodium of keratocytes exerts traction stress, \( \sigma v_r \), on the order of 100 Pa and have retrograde flow on the order of about 5 \( \mu m \) min\(^{-1} \) [67, 70], giving \( \alpha \approx 10^3 \text{ Pa s } \mu m^{-1} \). Taking an \( L_1 \) of approximately 2 \( \mu m \), and supposing the actin polymerization velocity is at least twice the retrograde flow velocity [67], \( v_p \approx 10 \mu m \text{ min}^{-1} \), gives an estimate of the membrane tension that stalls protrusion, \( \sigma_{mem(stall)} \), of \( \approx 200 \) pN \( \mu m^{-1} \). This value is in-line with keratocyte membrane tension (see table 1). Although this quantitative discussion is rather crude, and there is a large variability of parameters for different cell types, this calculation shows that cellular membrane tensions could certainly alter actin dynamics in the lamellipodium.

Given this potential effect of membrane tension on polymerization and/or retrograde flow, decreasing membrane tension could be hypothesized to increase cell protrusion [71]. Indeed lamellipodia extension speeds of fibroblasts are shown to increase when drugs or osmotic shock are applied that decreased tether forces, presumably reflecting a decrease in membrane tension [37]. During cell spreading, exocytosis keeps pace with the increasing surface area of the cell to maintain membrane tension constant, as mentioned above [39]. However when membrane tension is followed in real time, transient increases in tension are observed, which slow leading edge extension, until rectified by a burst of exocytosis [43]. Likewise if tension is increased artificially with hypo-osmotic shock, spreading slows.

However the relation between membrane tension and whole cell translocation is more complicated than the effect of tension on protrusion and spreading, as seen in a series of publications over the past 5 years [17, 27, 29]. When membrane tension is increased in nematode sperm cells, displacement speed is increased instead of the expected decrease [29]. Similar results are observed by a completely different experimental approach in human neutrophils [17].
both cases it is observed that cells are more streamlined in the direction of movement in higher membrane tension conditions. It is hypothesized that membrane tension helps organize protrusive forces by either inhibiting spurious protrusion away from the leading edge, or by causing protrusions to coalesce and work together, thus improving cell polarization (figure 3). Coalescence could be driven by the reduction in membrane deformation energy of combining two independent protrusions to one, illustrated by estimating the coalescence energy of actin-filled membrane protrusions. The energy of a single cylindrical protrusion of length $L_1$ and radius $r_1$ much larger than the natural radius of an empty membrane tether is on the order of $E_f(r_1) \approx 2\pi r_1 \sigma_{mem} L_1 \approx 10^4 k_B T$ (for $\sigma_{mem} = 100 \text{ pN} \mu\text{m}^{-1}$, $L_1 = 1 \mu\text{m}$ and $r_1 = 100 \text{ nm}$). The energy gain when two such protrusions coalesce into a single one of identical volume, containing the same number of filaments, is thus quite large: $\Delta E_f = (2 - \sqrt{2}) E_f \approx -10^4 k_B T$. Of course this energy estimate does not consider other factors, such as membrane anchoring to the cytoskeleton cortex, which could make coalescence less favorable. It does show however that membrane tension may play an important role in organizing cellular protrusions.

In keeping with the idea that membrane tension can drive coalescence of protrusions and thus enhance motility, when large amounts of membrane (cell area increases of over 70%) are delivered to moving keratocytes by liposome fusion experiments, movement is significantly hampered by the formation of multiple lamellipodia [27]. Multiple lamellipodia are also observed in low membrane tension conditions in protruding fibroblasts [37]. In other circumstances, where a keratocyte lamellipodium splits into two lobes upon encountering an obstacle (an atomic force microscopy tip), one of the two lobes eventually shrinks and reincorporates with the main cellular protrusion in a way suggesting an abrupt, mechanically induced slippage possibly triggered by an increase in membrane tension [47].

So overall it seems that for many different cell types and using varied manipulations, high membrane tension is associated with an organized lamellipodia, a single coherent structure with an unvarying polarity. Interestingly in the World Cell Race where the migration of multiple cell types along thin adhesive lines was examined, rapid movement was consistently associated with cells that did not switch direction [72]. Fast cells that lost polarity and moved in the opposite direction were not observed, meaning that high speeds and polarity maintenance are correlated. We can qualitatively understand why increased polarity gives increased speeds on the single filament level: a filament that is growing at some angle in relation to the direction of movement only contributes to forward motion by its projection onto the axis of movement $v_\parallel = \cos \theta v_p$, where $v_p$ is the polymerization speed [29]. It is less clear how membrane tension suppresses lateral protrusions and favors the main protrusion. One way of thinking about this is by treating the membrane tension as the load that is shared by the actin filaments that grow up against it. The force per filament is therefore inversely proportional to the local network density (see section 3.2), so in regions of higher filament density, like the leading edge of a cell, the impediment posed by membrane tension to filament growth is less than in sparser, lateral regions of the lamellipodium [12, 73]. The lateral regions are suppressed and the main protrusion is favored. This is also what is hypothesized to define the shape of the back of the cell [74].

In the preceding, we have considered that the lamellipodium is a flat, essentially two-dimensional structure. However the geometry of the cell leading edge membrane may also be important, not just its tension. When treated via classical hypo-osmotic shock to increase membrane tension,
keratocytes are observed to drastically increase their movement speeds, echoing results observed with C. elegans sperm cells [29,36]. At the same time, tether forces in shocked keratocytes remain unchanged, indicating that the keratocyte adjusts its membrane or cytoskeleton to maintain a constant membrane tension upon hypo-osmotic swelling, as seen in the liposome fusion experiments mentioned above [27]. Swelling is hypothesized to increase cell speed by increasing the contact angle the cell makes with the substrate, thus reducing the portion of membrane tension that is projected onto the growing filament ends and therefore reducing their load [36].

5. Outlook

Overall in the last few years, we have witnessed a change in how membrane tension is viewed in the context of cell motility and cytoskeleton organization. Recent studies have seen the convergence on a unifying theme: cells have less coherent and less directional lamellipodia and thus less efficient movement when under low membrane tension conditions. This is not to say that high membrane tension is always beneficial since high tension increases the cost of membrane deformation, affecting both polymerization-based and bleb-based protrusion machinery. The relationship between tension and motility might thus be biphasic, being optimal for intermediate tension values: at low tensions, cell protrusions are not polarized and are unstreamlined, while at high tensions, polymerization could be inhibited and/or retrograde flow could be increased (figure 4). The idea that is emerging is that the cell membrane acts as a long-range communication system between distant parts of the cell for cytoskeleton organization and polarity maintenance. The membrane can play this role because it spans the entire cell and changes in membrane properties can be transmitted rapidly, faster than the diffusion of signaling molecules through the cytoplasm. Confirmation of this idea will come with the development of better ways to measure membrane tension in vivo, for example fluorescent probes that give a read-out of membrane tension without changing it.

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