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# Biophysical Principles of Ion-Channel-Mediated Mechanosensory Transduction

**Charles D. Cox,[1,2] Navid Bavi,[3] and Boris Martinac[1,2,*]**
[1]Victor Chang Cardiac Research Institute, Lowy Packer Building, Darlinghurst, NSW 2010, Australia
[2]St. Vincent's Clinical School, University of New South Wales, Darlinghurst, NSW 2010, Australia
[3]Institute for Biophysical Dynamics, University of Chicago, Chicago, IL 60637, USA
*Correspondence: b.martinac@victorchang.edu.au
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**Recent rapid progress in the field of mechanobiology has been driven by novel emerging tools and methodologies and growing interest from different scientific disciplines. Specific progress has been made toward understanding how cell mechanics is linked to intracellular signaling and the regulation of gene expression in response to a variety of mechanical stimuli. There is a direct link between the mechanoreceptors at the cell surface and intracellular biochemical signaling, which in turn controls downstream effector molecules. Among the mechanoreceptors in the cell membrane, mechanosensitive (MS) ion channels are essential for the ultra-rapid (millisecond) transduction of mechanical stimuli into biologically relevant signals. The three decades of research on mechanosensitive channels resulted in the formulation of two basic principles of mechanosensitive channel gating: *force-from-lipids* and *force-from-filament*. In this review, we revisit the biophysical principles that underlie the innate force-sensing ability of mechanosensitive channels as contributors to the force-dependent evolution of life forms.**

Mechanobiology is an exciting, rapidly growing multidisciplinary research area at the intersection between biology, physics, engineering, and medicine. It studies the role that mechanical forces play in the physiology and pathology of biological systems by combining a large array of complementary methodologies (Roca-Cusachs et al., 2017; Lele et al., 2007; Sinha et al., 2018). Thanks to the new tools made available by recent progress in biophysics, molecular biology, genetics, computational modeling, and engineering research, mechanobiology has greatly contributed to our understanding of how mechanical environmental cues influence cytoplasmic and nuclear events. Major progress has been made in understanding how cell mechanics is linked to intracellular signaling and how this controls cellular behavior and fate (Discher et al., 2005; Iskratsch et al., 2014; Paluch and Discher, 2015). Given that mechanical force manifests itself in many forms, including the omnipresent gravitational and osmotic forces as well as contact-based sound, shear stress, stretching, bending, twisting, and compressing, it seems obvious that throughout evolution, different life forms have developed a multitude of ways to counteract and adapt to the mechanical challenges they continuously experience in their corresponding environmental niches.

There are different receptors at the cell membrane, including mechanosensitive (MS) ion channels, G protein-coupled receptors (GPCRs), and integrins. These receptors indirectly or directly interact with cytosolic force-sensing elements, such as microtubules and actin filaments. This way, they can sense and transmit biochemical signaling from the cell surface to the nuclear receptors that in turn may regulate gene transcription at the downstream level (Sinha et al., 2018). Among the primary mechanosensory membrane proteins at the origin of cellular signaling cascades, mechanosensitive ion channels are quite unique, as they are the most rapid in converting mechanical stimuli into electrochemical intracellular signals (e.g., on the millisecond timescale) (Martinac and Cox, 2017). These channels have been studied for >30 years and are widely expressed in both specialized sensory cells and non-specialized cell types. They have been cloned in all three domains of life encompassing prokaryotic and eukaryotic cells and organisms, including red blood cells (Hamill, 1983), chick skeletal (Guharay and Sachs, 1984) and frog muscle (Brehm et al., 1984), bacteria (Martinac et al., 1987), fungi (Gustin et al., 1988), and plants (Falke et al., 1988).

Functional mechanosensitive channels are pivotal in numerous mechanosensory transduction processes ranging from cellular osmoregulation in bacteria, fungi, and plants to the highly specialized senses of hearing and touch. As a result, mechanosensitive channels are present in both "specialized" cells dedicated to detecting exogenous cellular forces and non-specialized cells. Examples of specialized cells include those found in cutaneous sensory nerve formations, for example, Meissner's corpuscle (rapidly adapting mechanoreceptors) and Merkel cells (slowly adapting mechanoreceptors), which harbor Piezo2 channels and underlie the sense of fine touch (García-Mesa et al., 2017; Ranade et al., 2014; Woo et al., 2014; Maksimovic et al., 2014). Auditory hair cells, which are the site of the final mechanical transformer that underlies hearing, rely on functional transmembrane channel-like 1 (TMC1) and TMC2 proteins, whose genetic deletion renders hair cells of the knockout mouse mechanically insensitive (Corey and Holt, 2016). TMC1 and TMC2 seem to be part of a larger transduction complex, but alone or in combination, they seem to line the channel pore (Pan et al., 2018). Both members of the Piezo channel family are also important determinants of mechanosensing in specialized baroreceptive neurons that innervate regions such as the aortic arch (Zeng et al., 2018). Furthermore, the global deletion

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of Piezo1 was shown to be lethal in mice, indicating the importance of this mechanosensitive ion channel in mammals (Li et al., 2014). Moreover, mutations in mechanosensitive channels cause a plethora of hereditary diseases and contribute to the pathophysiology of many complex chronic conditions (Bae et al., 2013; Fotiou et al., 2015; Alper, 2017). (For a comprehensive review of the physiological roles of mechanosensitive channels, readers are directed to Martinac and Cox, 2017; Ranade et al., 2015; and Hamilton et al., 2015b.)

In addition to the specialized cells mentioned above, almost every biological system contains endogenous mechanosensitive channels, which are likely to be involved in general cellular functions such as migration and rigidity sensing (Discher et al., 2005). In this sense, all cells are mechanosensitive. This makes heterologous expression and characterization of new types of mechanosensitive channel proteins difficult (Dubin et al., 2017). In specialized mechanosensory systems, tissue tends to be scarce and the structural environment is equally as important as the identity of the mechanosensitive channel. Moreover, force application in experimental systems is not as readily controlled as ligand concentration, temperature, or voltage. Despite all of the difficulties in mechanosensitive channel identification, it is exciting to think that the genetic identity of a plethora of primary mechanosensitive currents in numerous organisms remains unsolved.

Central to these physiological functions is the ability of mechanosensitive channels to change conformation in response to mechanical deformation. In some of the earliest reports of mechanosensitive channel activity, it was immediately recognized that gating had to be controlled by membrane stretch (Sachs, 1988). When directly assessed whether pressure (stress normal to the plane of the membrane) or tension (stress in the plane of the membrane) gated the channels, it could be demonstrated in both whole-cell (Gustin et al., 1988) and the "excised patch" experiments (Sokabe et al., 1991) that membrane tension regulated mechanosensitive channel activity. This has justified the use of Laplace's law for a thin-walled sphere (e.g., cell membrane) at equilibrium, which links membrane tension T to pressure P and the radius of the membrane patch r through the expression $T = Pr/2$ (Sachs, 1986). The issue, however, has been in reconciling the fact that in eukaryotic cellular systems, the membrane tensions generated seem to be orders of magnitude lower (0.01–0.1 mN/m) than those experimentally measured to gate mechanosensitive channels in patch-clamp experiments (0.5–10 mN/m) (Lewis and Grandl, 2015; Maksaev et al., 2011). Apart from the relative simplicity of the assumptions made for theoretical and computational modeling of the mechanical properties of biological membranes (Bavi et al., 2014a; Rawicz et al., 2000; Rodowicz et al., 2010), this discrepancy could also be rooted in the fact that *in vitro* experimental force application (e.g., pressure clamp) is often very distinct from the way that these channels are tuned to sense mechanical stimuli in their native cellular environment. Two famous cases in point are how lipid bilayer asymmetry (e.g., due to its intrinsic pressure profile or asymmetric insertion of amphipaths) and local curvature can energetically shift the equilibrium state of MS channels in their native environment (Martinac et al., 1990; Guo and MacKinnon, 2017; Bavi et al., 2016b).

The aim of this perspective article is to review and update the biophysical concepts used to describe force sensing by mechanosensitive channels in cellular mechanotransduction. We also discuss a possible unifying principle that narrows the various concepts to a single physical force to which all possible forms of mechanical stimuli affecting cells and tissues are distilled down. Our aim is not to reduce these concepts to discussions of "spherical cows," but to propose a lowest common denominator of the mechanical stimuli acting at the cell membrane interface. In addition, we address how "force sharing" may explain some of the vagaries of mechanosensitive-channel-mediated mechanosensory transduction. We hope that a provocative discussion such as this will help lead to a better understanding of the evolutionary principles of mechanosensitive-channel-mediated mechanotransduction, anchored in the universal laws of physics and chemistry that have guided the force-dependent evolution of life forms.

## Life History: Strategies for Coping with Mechanical Stress

Mechanical forces act on the cell in numerous ways, including bending, compressing, shearing, twisting, or rupturing the membrane, cell wall, and/or cytoskeletal proteins (e.g., integrins, cadherins), depending on the force direction and intensity. To protect their structural and functional integrity, organisms belonging to different phyla on the evolutionary tree have developed different strategies to use these forces as physical cues and to reduce their potentially negative impact to a physiologically acceptable level.

### *Cell-Walled Organisms*

Cell-walled organisms, including prokaryotes (Bacteria and Archaea) and eukaryotic fungi and plants, are protected from sudden changes in environmental osmolarity by thick cell walls. Without the mechanical support of the cell wall, osmotic pressure would destroy the fragile cells due to water uptake upon a hypo-osmotic shock. Although the structure and structural components of the cell wall vary greatly among cell-walled organisms (Hamill and Martinac, 2001), they can on average resist osmotic pressures of up to 30 atm (Martinac and Kloda, 2003; Hamilton et al., 2015b; Peyronnet et al., 2014; Cox et al., 2018a). The thickness and Young's elastic modulus (a mechanical property indicative of stiffness of a solid material) differ between the cell envelopes of Gram-negative and Gram-positive bacteria. The apparent Young's modulus of Gram-negative bacteria such as *Escherichia coli* has been reported to be between 50 and 150 MPa, while it is estimated to be between 100 and 200 MPa for Gram-positive bacteria such as *Bacillus subtilis* (i.e., stiffer) (Tuson et al., 2012). The cell wall in Gram-positive bacteria is relatively thicker as well. Considering all of these physical properties, the *E. coli* cell wall can resist ~90% of the cellular turgor pressure, whereas the remaining 10% is sustained by the cytoplasmic membrane (Cox et al., 2018a). Given that the bacterial plasma membrane is supported by only a rudimentary cytoskeleton (Mayer, 2003), it is the lipid bilayer component of the plasma membrane that must resist most of the 10% share of the environmental osmotic pressure changes during hypo-osmotic shocks. This pressure share can correspond to an estimated membrane tension of ~18 mN/m, which approximates

the lytic tension of a pure lipid bilayer (~20 mN/m) (Nomura et al., 2012). As discussed briefly here and further on in the text, membrane tension of such magnitude is sufficient to fully activate bacterial mechanosensitive channels, such as MscL and MscS, which function as osmotic nanovalves (Levina et al., 1999; Cox et al., 2018a). Consequently, the peptidoglycan cell wall in bacteria protects the plasma membrane from excessive mechanical stimuli by taking the majority of mechanical forces affecting these organisms (Cox et al., 2018a). As demonstrated for MscS of *E. coli* (Shaikh et al., 2014), this channel exhibits increased mechanosensitivity in liposome patches compared to membrane patches of giant bacterial spheroplasts, which contain other membrane proteins; some of them are seemingly in direct contact with remnants of the cell wall present in giant spheroplasts.

MscL and MscS form separate subfamilies of prokaryotic mechanosensitive channels. The members of the MscL subfamily are largely confined to bacterial and archaeal cells, whereas members of the MscS subfamily are very diverse in terms of their structure and function, as well as their spread throughout organisms of different evolutionary origins, including algae, yeast, plants, and human parasites such as Plasmodium and Trypanosoma (Malcolm and Maurer, 2012; Cox et al., 2015; Haswell, 2007; Yoshimura, 1998; Zhou and Kung, 1992; Pivetti et al., 2003; Martinac and Kloda, 2003; Martinac et al., 2014; Martinac and Cox, 2017). In plants, for example, cellular turgor can be as high as 15–20 atm (Haswell, 2007; Peyronnet et al., 2014), and the resting membrane tension in plant protoplasts was reported to be ~0.12 mN/m (Morris and Homann, 2001), which is not enough to activate MscS channels. However, given that protoplast membranes were found to lyse at ~5 mN/m (Wolfe and Steponkus, 1983), temporary changes in the membrane tension of $\leq$5 mN/m are possible. Membrane tension of such magnitude is sufficient to activate MscS-like channels, which are thus likely contributors to the regulation of cellular turgor in plants similar to their function in prokaryotes. However, they have been found to play a role in more complex mechanotransduction processes, such as plastid morphology (Haswell and Meyerowitz, 2006) and pollen germination (Hamilton et al., 2015a).

### Animal and Human Cells

Compared to cell envelopes of the cell-walled organisms, the plasma membrane of animal and human cells is usually protected from large osmotic fluctuations and other excessive mechanical stimuli. These cells possess a cytoskeleton and extracellular matrix, which protect their cytoplasmic membrane while preserving cell deformability required for changes in cellular shape and size during cell growth, differentiation, and movement (Figure 1A). Both the extracellular matrix and the cytoskeleton are meshed and relatively soft structures, allowing the cells to modify their response to mechanical deformation (Elson, 1988). Due to differences in their structural components, the Young's modulus of animal and human cell membranes varies between hundreds of Pascals and several megapascals, depending on the cell and tissue type (Qi et al., 2015; Akhmanova et al., 2015). These cell membranes are thus much softer compared to the bacterial and fungal cell envelopes. Consequently, animal and human cells have developed different strategies to counteract excessive mechanical forces. They frequently contain excess membrane areas reaching up to 1,000% in the form of ruffles or invaginations, which by unfolding, can significantly reduce potentially deleterious effects of mechanical stimuli (Hamill, 2006; Erickson and Trinkaus, 1976; Hamill and Martinac, 2001; Sezgin et al., 2017). This type of mechanoprotection has also been suggested for caveolae (Lim et al., 2017; Golani et al., 2019).

In addition to their unique makeup and structure, living cells have developed robust closed-loop feedback systems to respond and adapt to their environments that are rich in mechanical cues that affect cellular signaling and communication. The forces sensed at the cell membrane interface are converted into an intracellular signal, which in turn instigates a cascade of downstream events. In some cases, these events can culminate in changes in gene regulation and protein expression. In this way, cells can progressively remodel their cortical cytoskeleton and cell membrane as a whole, allowing them to adjust to their mechanical niche until they are mechanically protected within the new stress limits (Luo et al., 2013). Given such robust closed-loop systems, mammalian cells could thus develop processes enabling them to grow, divide, and migrate to survive and protect themselves against excessive mechanical force (Grashoff et al., 2010; LeDuc and Robinson, 2007).

Although extensive emphasis in the literature has been placed on the cortical cytoskeleton and cell-adhesion molecules (e.g., integrins) as primary force sensors, mechanical stimuli must nevertheless be transmitted through the membrane lipid bilayer, which may be enough to activate mechanosensitive channels at the membrane interface. What is still unclear is the extent of mechanical stress developed in the membrane bilayer during different cellular processes and how mechanosensitive channels and plasma membrane crosstalk with both long-range and local forces stemming from the cytoskeletal cortex and extracellular matrix.

## Force Sensing at the Membrane Interface

The lipid bilayer forms the central core of the cell membrane to which both the extracellular matrix and the cytoskeleton tightly adhere. The cytoskeleton is anchored to the lipid bilayer via dynamic interactions between lipids, ankyrin, and actin-binding and integral membrane proteins. The tension generated within the cytoskeleton is not only focused on the cell cortex but is also transmitted by the highly integrated cytoskeleton network to the cell nucleus (Hamill and Martinac, 2001; Sinha et al., 2018). The solid part of the structure transmits mechanical deformation globally throughout the network from cellular focal adhesions (also referred to in the literature as "force foci"; Anishkin and Kung, 2013) around integrin heterodimers that function as transmembrane linkers between the extracellular matrix and the actin cytoskeleton (Ingber, 1997, 2006; Martinac, 2014).

Besides their attachments to the cytoskeleton, which vary from protein to protein, integral membrane proteins also intimately interact with the surrounding lipid bilayer (Laganowsky et al., 2014; Lee, 2004; Bavi et al., 2016a). Furthermore, it is believed that membrane proteins and lipids are organized into functional nano-domains, frequently referred to as lipid rafts (Simons and Toomre, 2000; Vereb et al., 2003; Anishkin and Kung, 2013; Sezgin et al., 2017), which enable selective and

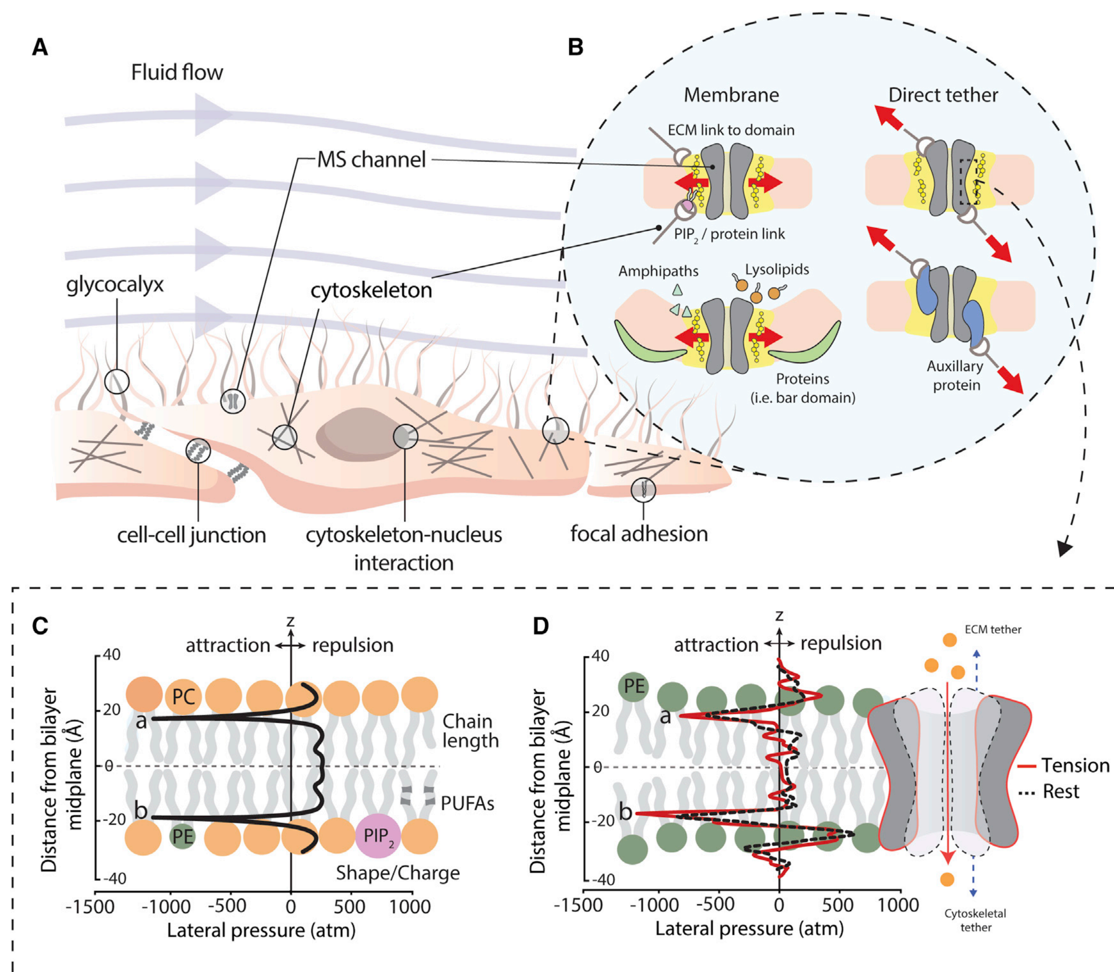


**Figure 1. Diagram of Cell Membrane and Gating Models for Mechanosensitive Ion Channels**

(A) Simplified structure of mammalian cell environment.

(B) Bilayer and direct tether models of mechanosensitive (MS) channel gating. Note how both can coincide where structural scaffold proteins focalize force to a mechanosensitive channel domain, but the final transducer is then the bilayer. This can be conveyed by links to structural proteins or lipids such as $PIP_2$. The curvature generated by proteins such as Bar domains (i.e., N-Bar) may also provide a stimulus to gate mechanosensitive channels.

(C) Idealized pressure profile from a symmetric bilayer. The profile can be modified by the properties of the embedded membrane proteins and constitutive lipid components.

(D) Calculated pressure profiles of the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) bilayer from molecular dynamic simulations at rest and with applied tension (Bavi et al., 2016b). Note the modification in pressure in the tail region and the increase in surface tension (peaks labeled a and b).

effective signal transduction from the cell surface to the cell interior. Like other membrane proteins, mechanosensitive channels are membrane constituents that contribute not only to the cell membrane structure and mechanical properties but also to its dynamics. Moreover, they have structurally evolved to sense and couple mechanical stimuli acting on cells and tissues to intracellular signaling pathways (Martinac and Cox, 2017). We discuss this issue further in the following sections.

## Mechanosensitive Ion Channels as Force Sensors

Mechanosensitive channels are ionotropic force sensors acting as primary transducers of mechanical stimuli. Given the complex membrane structures of organisms from all niches of life, resolving the role that the lipid bilayer, cytoskeleton, cell wall, and/or extracellular matrix play in mechanosensitive-channel-mediated mechanotransduction is one of the central questions awaiting definitive answers (Figure 1A) (Martinac and Cox, 2017).

What is known about MS channel activation and gating mechanisms comes mostly from studies of prokaryotic MscL- and MscS-like channels, as well as eukaryotic K2P-type (TREK1/2, TRAAK), transient receptor potential TRP (TRPV4, no mechanoreceptor potential C [NOMPC]—while many TRP channels seem to be involved in mechanosensory processes [Sharif-Naeini et al., 2009; Nikolova-Krstevski et al., 2017], whether they are mechanosensitive in most cases remains controversial), degenerin and epithelial sodium channel (DEG/ENaC), and Piezo1/2 ion channels (Hamill and Martinac, 2001; Gillespie and Walker, 2001; Martinac and Cox, 2017; Chalfie, 2009; Goodman and

Schwarz, 2003; Sachs, 2010; Sukharev and Corey, 2004; Honoré et al., 2015; Cox et al., 2015, 2016b; Ranade et al., 2015; Arnadóttir and Chalfie, 2010; Monshausen and Haswell, 2013; Yan et al., 2013; Hill and Schaefer, 2007; Knoepp et al., 2018b; Sharif-Naeini et al., 2008). At present, two basic models are used to describe the gating of mechanosensitive channels by mechanical force. The "force-from-lipids" model favors the view that force transmitted through the lipid bilayer directly gates the channels (Martinac et al., 1990; Teng et al., 2015; Kung, 2005; Markin and Martinac, 1991), whereas the "force-from-filament" model favors the notion of channel gating via cytoskeletal or extracellular tethers that are directly connected to the mechanosensitive channels (Figure 1B) (Chalfie, 2009; Katta et al., 2015).

### *Force-from-Lipids*

The force-from-lipids paradigm originates from experiments carried out first on bacterial mechanosensitive channels, which were exposed to a variety of amphipathic molecules to test whether the lipid bilayer could transmit the mechanical force required for mechanosensitive channel activation. Given that the effectiveness of the amphipaths tested was proportional to their lipid solubility, it was concluded that the mechanical force gating the channels could come from the lipid bilayer alone (Martinac et al., 1990; Markin and Martinac, 1991). In fact, in one of the first reports of mechanosensitive channels, Guharay and Sachs (1984) wrote, "We believe that the stretch response is due to a direct mechanical link between the s.a. *[stretch activated]* channel and the cell membrane." To confirm the force-from-lipids bilayer model and inherent mechanosensitivity of bacterial mechanosensitive channels (Markin and Martinac, 1991), they were purified and reconstituted into artificial lipid systems, which eventually led to the cloning of the first mechanosensitive channel, MscL (Sukharev et al., 1993, 1994, 1997). Using liposome reconstitution methods of purified mechanosensitive channel proteins as the ultimate reductionist approach, the inherent mechanosensitivity of all examined MscL- and MscS-like channels has been demonstrated (Kung et al., 2010; Martinac, 2011; Nomura et al., 2012; Moe and Blount, 2005; Kocer, 2010, 2015; Edwards et al., 2012). Liposomal reconstitution has thus become a gold standard for documenting the inherent mechanosensitivity of ion channels (Martinac et al., 2010). As the intimate interactions between lipids and integral membrane proteins become more evident, it becomes necessary to incorporate lipids of different charge, shape, and unsaturation to ensure a native-like environment for purified mechanosensitive channels.

In its essence, the force-from-lipids paradigm is an evolutionary conserved physicochemical principle that applies to cell membranes across all types of biological cells and can be used to explain why and how the mechanical force transmitted through the lipid bilayer alone can gate a mechanosensitive channel (Martinac et al., 2018). Force-from-lipids manifests itself in many ways that are not mutually exclusive. Two such examples are hydrophobic mismatch and bilayer curvature (Bavi et al., 2016c; Gullingsrud and Schulten, 2004; Perozo et al., 2002a, 2002b).

Given that the lipid bilayer is practically incompressible, membrane stretching thins the lipid bilayer, which results in hydrophobic mismatch between the hydrophobic length of the membrane spanning $\alpha$ helices of a channel and the bilayer. This will tend to minimize the mechanical strain on the bilayer by surrounding the channel with lipids of matching size and shape, which may influence helix tilt, and thus mechanosensitive channel gating, by shifting the equilibrium between open and closed conformations (Hamill and Martinac, 2001). Examples of mechanosensitive channels, whose gating was shown to be affected by hydrophobic mismatch include MscL (Perozo et al., 2002b; Nomura et al., 2012) and gramicidin (Martinac and Hamill, 2002). Bilayer curvature can be generated by the asymmetric incorporation of amphipaths into lipid bilayers, as indicated experimentally (Martinac et al., 1990; Nomura et al., 2012; Perozo et al., 2002a; Mukherjee et al., 2014) and theoretically using molecular dynamics simulations and analytical methods (Yoo and Cui, 2009; Meyer et al., 2006; Bavi et al., 2016b; Kheyfets et al., 2016; Maneshi et al., 2017). Thus, in addition to membrane stretch, local bilayer curvatures corresponding to a radius of curvature of $\leq$50 nm, which is comparable in size to mechanosensitive channel dimensions (Bavi et al., 2016b), provide an alternative mechanism for the activation of MS channels. The idea that curvature could be a relevant stimulus for mechanosensitive channels has been particularly well supported by the determination of the molecular structure of Piezo1, given that Piezo1 locally distorts the membrane forming local membrane curvature (Guo and MacKinnon, 2017). Whether this curvature changes during channel gating and the exact relevance of this curvature to cellular environments are unknown.

It has been argued that the force-from-lipids paradigm may apply only to bacterial mechanosensitive channels and their cousins found in archaea (Le Dain et al., 1998; Kloda and Martinac, 2001; Hamill and McBride, 1996; Matthews et al., 2007), fungi (Nakayama et al., 2012), and plants (Haswell, 2007; Haswell and Meyerowitz, 2006). The remaining question to be answered therefore is whether the force-from-lipids model can also apply to some or even all of the eukaryotic mechanosensitive channels. In recent years, evidence has shown that eukaryotic mechanosensitive channel proteins, including TREK1/2, TRAAK, Piezo1, and OSCA1.2, can also be gated by mechanical force in reduced systems consisting of lipids alone (Berrier et al., 2013; Brohawn et al., 2014a, 2014b; Cox et al., 2016a; Syeda et al., 2016; Murthy et al., 2018). While it is clear that mammalian membrane bilayers can sustain the large tensions required to gate bacterial channels such as MscL (>9 mN/m), including in the cell-attached configuration in the presence of an intact cytoskeleton (Cox et al., 2016a), recent work suggests that these forces are localized and not transmitted instantaneously as once thought (Shi et al., 2018). The question is whether under "normal" cellular conditions in which membrane tension is many orders of magnitude lower than this it could ever reach even 1 mN/m. As pointed out by others, determining the exact tension generated in the domain in which the mechanosensitive channel is positioned is a particularly difficult task (Sachs, 2015). Curvature is definitely a way to artificially magnify local membrane forces (Haselwandter and MacKinnon, 2018). This has the added bonus that curvature on this scale can be influenced by a host of proteins, lipid types, and potentially

circulating amphipaths (Figure 1) (Zimmerberg and Kozlov, 2006). Perhaps utilization of the new membrane tension probes that can be used *in situ* and in reconstituted cells can provide further answers to this fundamental question.

Although at the phenomenological level hydrophobic mismatch and local bilayer curvature differ, they are nevertheless only manifestations of the same physical force that is characteristic of biological membranes known as the transbilayer pressure profile. This is the universal effector of the force-from-lipids action on mechanosensitive channels. The transbilayer pressure profile is an intrinsic property of any lipid bilayer. It is a consequence of the ability of the lipid bilayer self-assembly and is characterized by large stress heterogeneity across the bilayer thickness (Cantor, 1999). The transbilayer pressure profile ranges from ~1,000 atm around the lipid head groups to hundreds of atmospheres around the bilayer center (Gullingsrud and Schulten, 2004). Although the transbilayer pressure profile has usually been determined using computational simulations (Gullingsrud and Schulten, 2004; Bavi et al., 2016a, 2016b) attempts have been made to measure it experimentally (Templer et al., 1998; Ridone et al., 2018). The calculated values of the intra-bilayer pressure range between ~250 atm for monounsaturated (18:1) lipids to ~350 atm for polyunsaturated (18:2 and 18:3) lipids, which are in very good agreement with pressures calculated analytically (Cantor, 1999; Gullingsrud and Schulten, 2004) and with pressures determined recently by NMR spectroscopy (Ridone et al., 2018). These measured changes in the transbilayer pressure profile influence both MscS and MscL reconstituted into liposomes made of these specific lipid types. Further support for the above conclusion follows from comparing transbilayer pressure profile anisotropy in an idealized lipid bilayer composed of two identical monolayers with the pressure profile of the same bilayer upon insertion of a membrane protein. Transbilayer pressure profile of a pure bilayer is symmetric, exhibiting in each monolayer negative pressure peaks resulting from the surface tension at the water-lipid interface and repulsive positive pressure peaks at the lipid head groups and hydrophobic tails (Figure 1C). To balance the forces between the monolayers upon insertion of a membrane protein such as a mechanosensitive ion channel, the pressure profile between the monolayers becomes asymmetric, depending on the shape of the inserted protein (Figure 1D). Exogenous force application and further modification of the transbilayer pressure profile asymmetry can then influence the conformational dynamics of a mechanosensitive channel.

Another way to modify transbilayer pressure profile asymmetry is by the unilateral insertion of amphipathic molecules such as conical lipids (e.g., lysophosphatidylcholine) and small molecules, including chlorpromazine and local anesthetics. The effect of amphipaths on bacterial mechanosensitive channels has been well established (Bavi et al., 2014b; Kloda et al., 2007; Markin and Martinac, 1991). These compounds (Martinac et al., 1990; Perozo et al., 2002b), depending on their lipophilicity and shape, may affect local bilayer curvature, but at the very least they induce frustration within the bilayer. Besides the compounds mentioned, there is an almost never-ending list of amphipathic molecules produced and released in the physiological setting that could act to modify mechanosensitive channel function without specific protein-binding sites. The mechanism of how many amphipaths may activate mechanosensitive channels remains unknown (Yoo and Cui, 2009). If local curvature is required, then only local curvatures comparable in size to mechanosensitive channel dimensions (<50 nm in radius) can induce a change in the transbilayer pressure profile asymmetry of several millinewtons per meter, which are sufficient to activate the mechanosensitive channels (Bavi et al., 2016b). The unilateral insertion of amphipaths into the lipid bilayer causes a change in the pressure profile asymmetry, which is strongly dependent on the structure of the affected mechanosensitive channel (Clausen et al., 2017). We have seen this most strikingly with the amphipathic compound 2,2,2-trifluoroethanol (TFE), which shows asymmetric effects on prokaryotic and eukaryotic mechanosensitive channels (Bavi et al., 2017). Potentially, the asymmetric insertion of amphipathic compounds into lipid bilayers is physiologically relevant to several eukaryotic mechanosensitive channels—for example, the effect of lysophosphatidylcholine (LPC), arachidonic acid, and lysophosphatidic acid on the activity of $K_{2P}$ channels (TREK and TRAAK), *N*-methyl-D-aspartic acid (NMDA) receptors, and more recently, the effect of β-amyloid and lysophosphatidylserine on Piezo1 channels (Patel et al., 1998; Kloda et al., 2007; Syeda et al., 2016; Tsuchiya et al., 2018; Clausen et al., 2017). However, it has yet to be conclusively shown whether their effect is through their specific interactions with the protein or through perturbation of the transbilayer pressure profile.

Given the universal role that the lipid bilayer plays in mechanosensitive channel function, the question becomes, is there any common mechanism for lipid-bilayer-mediated mechanosensitivity? Despite little to no structural and sequence similarity, eukaryotic mechanosensitive channels are functionally very similar to prokaryotic MscL- and MscS-like channels. In other words, many lessons that have been learned from the gating mechanisms of such unique bacterial channels may be extended to mechanosensitive channels in eukaryotes. Based on the gating mechanism of MscL, it has been proposed that for force-from-lipids to effectively gate a mechanosensitive channel, there should be a part of the channel that acts as a sensor by intimately interacting with the lipid bilayer (e.g., MscL N terminus), while being directly connected to a pore-forming unit of the protein (e.g., TM2 in MscL) (Bavi et al., 2016a; Iscla et al., 2008; Kung, 2005; Teng et al., 2015; Martinac et al., 2018). Recent structural evidence from Piezo1 channels and functional data from TREK-1 and OSCA1.2 channels support this idea (Honoré et al., 2006; Saotome et al., 2018; Maity et al., 2019). However, proposing such a mechanism by no means excludes the possibility of long-range allosteric interactions within the protein structure, which may also regulate channel function. Rather, it points to the most efficient, hence common, way of force transmission to the main unit of a channel protein (i.e., the pore region). Of course, these structural and mechanistic similarities exist in other channels, where instead of force-from-lipids, the gating stimuli can be membrane potential, pH, or ligand binding.

### *Force-from-Filament*

Force transmission absolutely requires different cellular structures that, besides membrane receptors embedded in the lipid bilayer, includes the extracellular matrix and cytoskeleton (Pruitt

et al., 2014; Martinac, 2014; Sheetz and Yu, 2018) (Figure 1). The role that the extracellular matrix and cytoskeleton play in the direct activation of mechanosensitive channels is nicely illustrated by the following two examples.

NOMPC, the founding member of the TRPN subfamily of ion channels, is essential for mechanosensory responses in *Drosophila* (Walker et al., 2000). The members of this family are generally found in invertebrates and only in some lower vertebrates (Kang et al., 2010; Shin et al., 2005). NOMPC contains 29 ankyrin repeats acting as a tether that couples the N terminus of the channel with microtubules attached to the plasma membrane. NOMPC is activated by mechanical force pulling on microtubules via the ankyrin tether (Zhang et al., 2015). The same study also showed that equipping a non-mechanosensitive $K^+$ channel with the NOMPC ankyrin repeats converted it into a mechanosensitive channel, which provides strong evidence for the force-from-filament gating as a complementary mechanism for mechanosensitive channel activation. However, the participation of the lipid bilayer in NOMPC mechanosensitivity cannot be completely ruled out (Hardie and Franze, 2012), and the potential relevance of force-from-lipids for NOMPC gating is addressed in the next section.

Another example is ENaC, the epithelial $Na^+$ channel, which responds to shear forces in endothelial cells from different vascular beds (Knoepp et al., 2018a). The shear force-sensing mechanism has been probed, and convincing evidence suggests that the pore-forming α subunit of ENaC is in direct contact with the extracellular matrix via two glycosylated asparagines. This direct connection of αENaC with the extracellular matrix seems to be crucial for shear force sensing and in turn its role in blood pressure regulation (Knoepp et al., 2018b). In addition, this provides a clear example of how post-translational modification could be essential to the mechanical gating of some mechanosensitive channels and may confound heterologous expression. In fact, ENaC activity also seems to be dependent on palmitoylation or S-acylation (Shipston, 2011), the reversible process whereby a lipid chain is covalently bound to a cysteine residue. Applying force via a covalently bound lipid seems like the most efficient form of force-from-lipids. It will be interesting to see whether other eukaryotic mechanosensitive channels are palmitoylated and whether this influences their mechanosensitivity.

In addition to the above-described NOMPC and ENaC, mechanosensitive channels in specialized mechanosensory cells such as hair cells are currently thought to be activated via tethered mechanisms (Martinac, 2014; Martinac and Cox, 2017; Bechstedt and Howard, 2007; Corey and Hudspeth, 1979), although here it is important to note that there is still heavy interplay and dependence on the surrounding bilayer (Peng et al., 2016; Effertz et al., 2017).

It is not only structural proteins that modify force transduction, however. There is lateral heterogeneity in the membrane in the X-Y direction in the form of the highly ordered and dynamic "raft domains," also referred to as "lipid microdomains" (Nicolson, 2014), occurring on the scale of tens to hundreds of nanometers alongside less organized and more fluid regions (Sezgin et al., 2017). While the existence of separate but temporally dynamic domains formed by lipids and proteins of specific geometry and interfacial interactions is largely undisputed, the exact nature of such dynamic entities is much more open to interpretation. It also remains unknown whether different mechanosensitive channels localize in such domains, and if so, what type of domain they energetically select. For mechanosensitive channels, either such domains confine and restrain their movement or they act as "stiffened platforms" for the efficient transmission of mechanical force (Anishkin and Kung, 2013). Also, how the perturbation of these microdomain components and dynamics (e.g., through the modulation of lipids, proteins, or mechanical force) could affect mechanosensitive channel function is another important biophysical question. A recent kinetic model of mechanotransduction addressing this question proposes that a mechanism based on the disruption of ordered lipids could initiate a mechanosensitive signal for the activation of phospholipase D2 (PLD2) (Petersen et al., 2016). According to this mechanism, mixing PLD2 with its substrate resulting from the disruption of ordered microdomain components produces phosphatidic acid, which serves as a signaling lipid molecule in many biological processes (Wang et al., 2006). It is worth mentioning that Piezo1 reconstituted in asymmetric droplet bilayers doped with 1,2-dioleoyl-sn-glycero-3-phosphatidic acid [DOPA] in one monolayer was constitutively active (Coste et al., 2012), which resembles the activation of bacterial mechanosensitive channels by amphipaths according to the force-from-lipids principle (Martinac et al., 1990; Teng et al., 2015). In our view, any of these particular mechanisms of mechanotransduction, be it membrane tension, shear force, curvature, or disruption of the ordered lipid microdomains, would result in a change in the transbilayer pressure profile asymmetry that, as discussed above, is the universal effector of the force-from-lipids mechanism of mechanotransduction at the membrane interface.

We have gained some insight into how the tight yet dynamic interactions of lipids with mechanosensitive channels at their transmembrane periphery regulate their function and oligomerization (Pliotas et al., 2015; Laganowsky et al., 2014). Nevertheless, several biochemical (lipid and protein) spectroscopic assays and high-resolution microscopic approaches will be required to simultaneously address mechanosensitive channel dynamics while interacting with annular lipids and auxiliary proteins.

### *Force Sharing*

The robust examples of NOMPC and ENaC showing that eukaryotic mechanosensitive ion channels do use molecular tethers for their activation argues against a common denominator in mechanosensitive channel activation. In fact, neither NOMPC nor ENaC have been shown to be gated directly by bilayer forces. However, these examples still provide a battery of information to suggest that force-from-lipids and tether-based gating paradigms may not be mutually exclusive. It is patently clear that stress applied to a cell membrane is always shared between the cytoskeleton and/or extracellular matrix and membrane proteins, which are directly exposed to the mechanical properties of the lipid bilayer in which they are embedded. Force-sharing models of mechanosensitive channel gating in animal and human cells have already been proposed (Kung, 2005; Chalfie, 2009; Martinac, 2014; Gaub and Müller, 2017),

and we believe that NOMPC is a good example to illustrate this. Mutation of His1423 to Ala in the S4-S5 linker of NOMPC renders the channel no longer responsive to mechanical stimulation (Jin et al., 2017). Since it seems that His1423 residue in NOMPC interacts directly with the lipid bilayer, this specific lipid-protein interaction is also likely to be critical for activation by mechanical stimuli. This proposal seems plausible given the role that horizontal membrane-coupling helices, such as the S4-S5 linker of NOMPC, play in other mechanosensitive channels (Bavi et al., 2016a; Cox et al., 2016b; Maity et al., 2019). How membrane tension is modulated by cytoskeletal proteins is thus an exceedingly relevant question.

The tension threshold for the activation of Piezo1 in cell membrane blebs as well as in pure lipid bilayers was reported to be ~1–3 mN/m (Cox et al., 2016a; Syeda et al., 2016; Lewis and Grandl, 2015). In fact, in the presence of STOML3 and tropomyosin 4.2, we see that the membrane tension required to gate Piezo1 is $\leq$0.5 mN/m, indicating that these cytoskeletal-associated proteins sensitize Piezo1 to mechanical forces within the physiological range of the membrane tensions that are observed for mammalian cells (Poole et al., 2014; Gauthier et al., 2012). Furthermore, Piezo1 also becomes ~10 times more sensitive to mechanical pulling when adhering to extracellular matrix proteins such as collagen IV (Gaub and Müller, 2017). In contrast the actin cross-linker filamin A increased the tension threshold for the activation of the channel in accordance with its role in arterial remodeling (Retailleau et al., 2015). Thus, we would advocate a scenario in which Piezo1 gating follows the force-from-lipids paradigm, but that extracellular matrix and cytoskeleton can markedly affect the forces detected by this membrane-embedded ion channel protein. In fact, MscL of *E. coli* expressed in HEK293T cells required higher pressures to gate in excised inside-out patches from cells than in cell blebs, thus further confirming the regulation of tension-gated ion channels by the cytoskeleton (Cox et al., 2016a, 2018b). These results demonstrate that both the extracellular matrix and cytoskeletal proteins can dynamically regulate the activity of inherently mechanosensitive bilayer-gated channels. Although estimates of the tension sensitivity of Piezo1 in droplet interface bilayers suggest that Piezo1 requires 1–3 mN/m to gate, this is hampered by a lack of knowledge of the exact area of the contact between the droplets, so these values could in fact be lower (Syeda et al., 2016). A further conundrum has been the inability to gate Piezo2 channels by stretch, and we await answers as to why this channel is not gated in classical cell-attached patches.

## Conclusions and Perspective

Our understanding of the biophysical principles of mechanosensitive-channel-gating mechanisms has significantly increased over the last decade due to the work showing that eukaryotic mechanosensitive channels TREK-1/2, TRAAK, Piezo1, and OSCA1.2, like the bacterial MscL and MscS channels, exhibit inherent mechanosensitivity according to the force-from-lipids principle. We believe that electrophysiological recordings from these mechanosensitive channels reconstituted into liposomes and planar bilayers combined with fluorescence microscopy, electron paramagnetic resonance (EPR), NMR spectroscopy, and computational methods have revealed that the force-from-lipids principle originates from changes in the asymmetry of the transbilayer pressure profile. The sensitivity to these changes is then determined by the shape of the mechanosensitive channel protein and its specific interactions with the cytoskeleton and/or the extracellular matrix. While many channels can function in reduced systems consisting of only protein and lipids, the next question is whether this is the preferred mechanism of force transduction in the cellular environment. For example, while Piezo1 may open in reduced systems and has the ability to act as an inherent mechanosensor, is there a necessity for molecular tethers for *in vivo* function? In particular, the amount of force sharing between the bilayer and cytoskeleton and/or extracellular matrix and how this may sensitize or desensitize mechanosensitive channels requires further study. Despite these outstanding questions, the current picture supports the force-from-lipids principle as the fundamental physicochemical principle and unifying paradigm of mechanotransduction at the membrane interface.

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## AUTHOR CONTRIBUTIONS

All of the authors contributed to the writing of the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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