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Mechanical Transduction and the Dark Energy of Biology

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Cells have three sources of energy: chemical, electrical, and mechanical. We know a fair amount about the first two, and very little about the third. The study of mechanical transduction is difficult because the stimulus at the site of a transducer (in this case an ion channel) is generally unknown (1,2). The mechanics of cells involves the interaction of many structural components including the cytoskeleton, the lipid bilayer, and the extracellular matrix and intracellular organelles (3). This sharing of stress means that a mechanical stimulus supplied by an experimenter is split between many components, only some of which may affect the channels. The best data to date suggests that mechanosensitive ion channels (MSCs) respond primarily to tension in in the bilayer (4–8), although channels in specialized sensory organs may utilize a direct linkage to the cytoskeleton (9,10). Patch experiments have shown that stress in the bilayer is comparable to the stress in the cortical cytoskeleton (11), but both of the components are viscoelastic and often plastic, hence the forces directly acting on MSCs are not accurately defined. Even channels reconstituted in lipid bilayers have protein-bound lipids that effectively alter the line tension of the domain so the magnitude of the local stimulus is different from that predicted from the macroscopic stimulus parameters (12). This review focuses on the factors that affect the stimulus at the channels, but does not deal with the molecular structure or the genetics of MSCs; note that there are many such reviews available (5,9,13–25).

Let us consider different types of mechanical stimulation (2). The most common stimulus is a patch pipette where the applied pressure is varied to change tension in the cortical membrane of the patch. As is well known, tension in the bilayer is shared with the proteins of the cortical cytoskeleton (11). The patch cytoskeleton is different from that of the cell it was taken from (26). The forces involved in patch formation disrupt the normal cytoskeleton, yielding new cortical structures. The adherence of the patch to the walls of the pipette creates the gigaseal but also generates large tensile forces (~3 mN/m) in the patch so that all published patch recordings are made under conditions of high membrane stress. The stress in resting cells is very small: 0.02–0.04 mN/m (27). The stress in patches is variable in space and time (26) even if the applied pressure is constant, so we should expect nonstationary scatter in patch data. The unknown time dependence of local stimulus complicates kinetic studies of the channels. How much of a channel's activation or inactivation or adaptation kinetics reflects relaxation of the local stimulus? How much of the kinetics depends on the flow of the lipids (28)? Let us consider a system other than a patch.

Channels in a reconstituted planar membrane are under considerable resting tension due to adhesion of the lipids to the Plateau-Gibbs border where lipids meet the supporting structure (29). It is not possible to observe channel kinetics at low tension in a planar bilayer because the bilayer tension is buffered by the Plateau-Gibbs border, which also means that bilayer bending with hydrostatic pressure does not increase the bilayer tension (30). Reconstitution into lipid vesicles avoids the problems of membrane binding to the glass, but studying patches made from vesicles reintroduces those stresses (31–33). Whole-vesicle patching removes those problems because most of the membrane that contributes current is not close to the pipette (34).

Another popular mechanical stimulus is osmotic pressure, which assumes that osmotic stress is borne by the cell's cortex. However, in general, this is not found to be true, because most cells are filled with a cross-linked gel-like cytoskeleton that bears most of the stress, much like a sponge (35–37). AFM experiments have shown that cells with a 3D cytoskeleton get softer with swelling, which is in contradiction to the

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cortical stress model (38). Direct measurements of tension in cytoskeletal proteins in hypotonically swollen cells show that the stress is distributed and not concentrated in the cortex (39–42). If one is working with a cell lacking a full cytoskeleton, such as a red blood cell, then the cortical tension will increase with osmotic pressure. Equivalently, if the bilayer separates from the deeper cytoskeleton as in a bleb (43), then bleb tension will increase with osmotic pressure. The tension in a cell's bilayer due to osmotic pressure is unknown although it is probably monotonic with cell volume.

The bilayer contains many domains with different physical properties, so where the channels are located will affect their mechanical sensitivity. Electrophysiology data of Piezo1 show the presence of functional domains. We found that we could record currents from many channels in a patch, and with repeated stimulation the channels lost inactivation, not one by one, but all at once. How do many channels communicate simultaneously? One method is if they were driven through a domain boundary that could fracture (44) (Fig. 1). We found that channel activation was unaffected by a putative fracture, but inactivation was affected and seemed to require that the channels be close to each other. When the channels are physically separate, as in reconstitution experiments, they do not inactivate; therefore, inactivation is not an intrinsic state of the protein, but it does seem to require channel/channel interactions. Activation of WT human Piezo1 has no measurable latency to the generation of current, so there are unlikely to be many subreactions in the opening process. However, we have found that mutations that slow inactivation often also produce random latencies to activation (45,46). What is going on during the latency is unknown, although it may reflect the time required for a domain to fracture.

How can you measure the bilayer tension (not cortical membrane tension) in a patch? The best solution we have found is to use a cotransfected precalibrated ion channel. We used the eukaryotic-expressing bacterial channel, MscL (47), that has been calibrated in lipid membranes to have a Boltzmann relationship between open probability and tension (4,48,49). The midpoint of that curve is ~11 mN/m (4,49). We cotransfected HEK cells with a noninactivating mutant of human Piezo1 and MscL and varied the patch suction to vary the tension and traced out a dose-response curve of P_{open} versus pipette pressure for both sets of channels (44). If we assume that both channels, Piezo1 and MscL, are in similar bilayer domains, we then extract the free energy between closed and open. Approximating the channel as a right circular cylinder embedded in the bilayer, the energy between closed and open would be $\Delta G = T\Delta A$, where T is tension and ΔA is the change of in-plane area between closed and open. This calculation suggests that MscL has an ΔA value of ~20 nm^2 in agreement with published data (22). Surprisingly, hP1 had a similar ΔA, despite a radically different structure. As a control, we measured ΔA for the K^+-selective MSC, TREK-1, and this was only ~10 nm^2 (44).

Piezo is the largest known membrane protein, but a large size is not necessary to make an MSC; e.g., TREK is much smaller than Piezo. So why did nature make Piezo into such a large structure? Possibly it was to intertwine the channels to form domains (Fig. 2). Or, because the channel has such a wide span, ~100 nm, the channel may become sensitive to local curvature (50–52). Does curvature matter? In experiments we did with TREK-1, we found that we could create changes in local patch curvature with a sudden change from

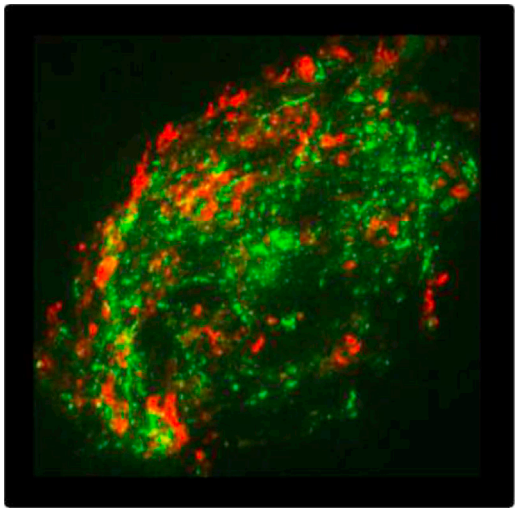

FIGURE 1 MSCs form discrete domains. Given here is a single HEK cell cotransfected with Piezo1 in red and TREK-1 in green. Both types of channels form domains that are separate and the tension in each type of domain may be different. To see this figure in color, go online.

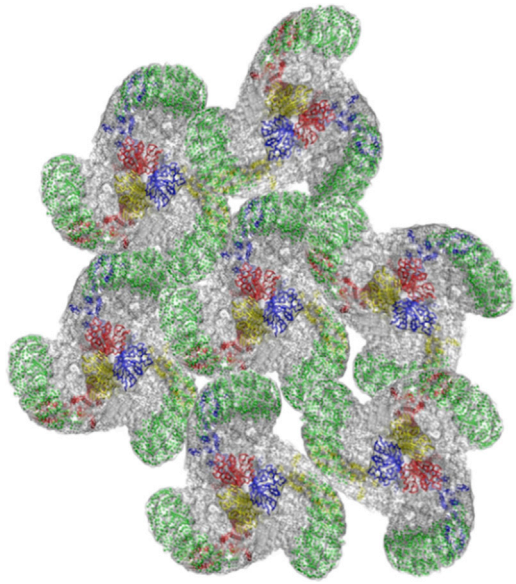

FIGURE 2 Putative model of a Piezo domain formed by bonding of channels to each other. Individual channel images are modified from (1). To see this figure in color, go online.

negative pressure to zero pressure (7). Under suction the patch area is close to a spherical cap with an area of $2\Pi r^2$, and when the pressure goes to 0 mmHg, the equilibrium patch geometry is close to a planar disk of area Πr^2 (33). This transient excess area of Πr^2 caused a wrinkling of the patch that produced no change in TREK-1 current (7). The wrinkles disappeared as the membrane spontaneously reannealed to the pipette in ~0.5 s. The wrinkles appeared to be folds <0.5 μm wide with a radius of curvature at the vertex that was much smaller than the mean dome curvature (7) (Fig. 3). For curvature to be an effective mechanical stimulus, the radius of curvature should be comparable to the channel dimensions (53,54). Syeda et al. (55) estimated a gradient of tension across a bent bilayer in their supporting material.

This wrinkling idea makes me think of the effect of positive and negative patch pressure. There are reports of rectifying gating that only works with positive or negative pressure (56,57). When an excised or cell-attached patch is exposed to positive pressure, the membrane peels off the wall, forming a spherical cap that points toward the pipette tip (Figs. 3 and 4). However, at the junction of the membrane with the glass, there is a very tight radius of curvature. We do not know where the active channels are located in a patch; could they be in the bends where the membrane meets the glass?

Physiological roles of MSCs

There are many physiological processes that involve mechanical transduction: blood pressure regulation, filling of other hollow organs (58,59), proprioception (60), touch (61,62), and hearing (63,64). There are undoubtedly many more processes involving MSCs, because any process that involves changes in cell shape involves change in force, notably in development (65). In prokaryotes, MSCs serve to regulate osmotically active solute concentrations, but eukaryotic MSCs are cation-selective (66–68), so by themselves they cannot regulate volume. In red blood cells, MSCs are sensitive to the specific inhibitor, GsMTx4 (44,69–71). In sickled cells, crystals of hemoglobin may expand sufficiently to press against the membrane, thereby activating MSCs and generating GsMTx4 sensitivity. MSCs have a strong influence on blood vessel function (72–74). Also involving mechanical stress, GsMTx4 is a potent stimulator of neurite growth, probably by inhibiting Ca^{+2} entry (75,76).

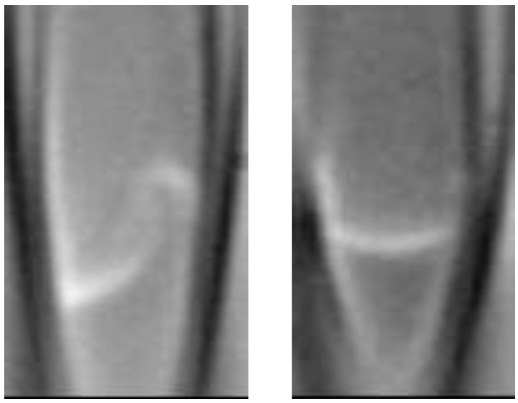

FIGURE 3 Two differential interference contrast microscopy images of patches in a pipette. In the left panel, the change in curvature emphasizes the presence of mechanical domains in a patch. In the right panel, an inside-out patch has been pushed toward the tip with positive pressure. Note that on the left side, the membrane has been partially peeled off the glass, and there is a kink in the membrane where the fold occurs. These are images from a video; in steady state, the local curvature tends to disappear (2).

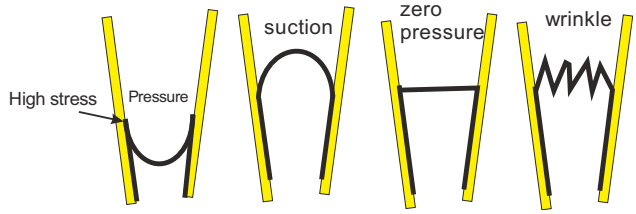

FIGURE 4 Cartoon of patch geometry under different conditions. Positive pressure generates high curvature where the membrane leaves the glass and begins pointing downward. Because dome tension is symmetric with respect to pressure, there seems to be no intrinsic way for channels in the dome to sense a particular sign of the pressure. Images of inside-out patches revealed that positive pressure peels the membrane off the wall (4,26). The channels that are seen with positive pressure may represent channels activated in the region of extreme curvature, such as where the bound membrane is peeled off the glass and pushed toward the tip (33) (Figs. 3 and 4). It is also possible that the enormous stresses caused by positive-pressure extrusion rip loose the cytoskeleton to transfer local mean tension to the bilayer. To see this figure in color, go online.

Biophysics of the channels

For a channel to be mechanosensitive, it has to change dimensions under stress. Any membrane-bound protein that changes shape will be sensitive to changes in membrane tension. The voltage-sensitive channels such as K^+, Na^+, and Ca^{+2} are all modulated by membrane tension (77–81), as is the NMDA channel (82). Using the term "mechanical modulation" suggests that mechanics cannot transmit enough energy to cover the whole dynamic range of a channel but can alter rates (22). To simplify discussion, I will refer to MSCs as those channels that can be driven over their whole dynamic range with mechanical stress alone.

The simplest model for the energy of gating is to treat the channel as a right circular cylinder in the membrane with two states (closed and open) with different in-plane areas (25). The energy difference between closed and open states is then $\Delta G = T\Delta A$, where T is the tension in the bilayer and ΔA is the difference of in-plane area between closed and open. For a given tension, larger changes in area produce higher sensitivity. However, there are other potential energy

terms. For example, if the channel changes thickness upon opening, the bilayer and the channel thickness may deviate, so energy should be added or subtracted from the reaction. This process is called "hydrophobic mismatch" (83–85). AFM data suggests that if a channel like *Shaker* opens as a wedge, with the cytoplasmic monolayer expanding more than the extracellular monolayer, it causes buckling of the bilayer—thus contributing mechanical potential energy to the reaction (51,86). Changes in membrane curvature can drive gating (87), and this is particularly relevant to endo- or exocytosis. Curvatures of 1/40 nm^{-1} have been shown to add $\sim 7\ k_BT$ to a channel relative to the flat membrane (87). More differentiated MSCs can be linked in series with elements of the cytoskeleton, and this seems to be characteristic of sensory organs (10).

Pharmacology

MSCs can be inhibited by Gd^{3+} (88–91), but that is a highly nonspecific inhibitor (91). It may inhibit by condensing the negative lipids (89) and reducing the free volume of the bilayer (92). The only known specific inhibitor of any MSC is GsMTx4 (93), a 34-amino-acid ICK peptide found in tarantula venom (94), which was later synthesized (95). We have shown that the D- and L-forms of GsMTx4 are equally active, suggesting that there are no protein-protein interactions involved (95). The interaction of GsMTx4 with lipid membranes shows an association at the extracellular aqueous boundary (96,97). Recent unpublished results using an environmentally sensitive, fluorescently labeled GsMTx4, suggest that the peptide does not enter the bilayer at resting tension, but enters with increased tension (T.M. Suchyna, personal communication). The resulting increased free volume with an increase of tension provides space for peptide penetration. The space taken up by the inserted GsMTx4 compresses the surrounding lipids, causing a drop in local tension. At saturating concentrations ($\sim 10\ \mu M$), GsMTx4 is a gating inhibitor equivalent to an offset of ~ 60 mmHg of patch pressure. Because GsMTx4 does not appear to act on membranes at resting tension, its effect as an inhibitor can only be tested when the membrane is under pathologic stress, i.e., when MSCs are active.

In situ experiments suggest that GsMTx4 has (98) minimal side effects on animals despite its wide efficacy in a variety of in vitro systems (14). This lack of toxicity in situ may represent the inability of GsMTx4 to bind to resting membranes. Its effects are confined to cell membranes under pathologic tension. In dystrophic skeletal muscle cells, GsMTx4 protects against the loss of contractile strength and rundown with repeated stimulation (99). Its use as a drug for dystrophy is further assisted by the fact that the pharmacologic half-life (in mice) is ~ 1 week, and it does not penetrate the blood brain barrier. However, like many other drugs, GsMTx4 binds to blood plasma proteins so if intravenous administration is used, blood proteins will bind a good deal of the applied drug (76). That partitioning may explain why recent experiments on the effect of GsMTx4 on cardiac arrhythmias in pigs found little effect (100). In contrast, the cardiac sensitivity to GsMTx4 in saline was demonstrated in Langendorff rabbit hearts, where it reversibly suppressed inflation-stimulated atrial fibrillation at ~ 170 nM (101). GsMTx4 has shown no toxic effects on human cardiac muscle (102) or in free roaming ferrets (98). Mild side effects of GsMTx4 at high concentrations have been seen in hair cells (63,103) and possibly TRPC channels (104).

MSCs are traditionally sensitive to amphipaths (19,47,95–97,105–115). The amphipath effects seem to be local to the channel (say within three lipids of the protein) rather than correlated with changes in global bilayer properties. Lots of drugs in clinical use are amphipathic, so some of their side effects could be caused by interactions with MSCs. An interesting aspect of this amphipath sensitivity is that general anesthetics activate the 2P MSCs in the concentration ranges used for clinical dosing (111,116). We might speculate that these channels are the responsible for people getting knocked out by a blow to the head; the 2P channels are activated by mechanical stress, and that leads to hyperpolarization of neurons with a resulting loss of excitability. As the channels inactivate (7), normal operating voltages return and the client wakes up.

Cautions

Although Patapoutian's lab originally found Piezo in N2A cells (117), others have found no activity in WT N2A cells (118). Variable expression was also emphasized by the report that HEK cells had little MSC activity (67), despite the fact that we cloned human Piezo1 and 2 from HEK cells (119). The presence of channel RNA clearly is not equivalent to the functional expression of channels. A key surprising observation affecting the interpretation of physiological effects of MSCs stems from the work of Lauritzen et al. (120). They showed that expression of 2P channels had gross effects on structure of the actin cytoskeleton, even if the channels were nonconducting. We have seen similar histological effects. Changes in the cytoskeleton will affect tension in the bilayer, so we expect that any drugs affecting the cytoskeleton will also affect MSC activity (121–123). We and others (124) have also seen strong correlations between MSC activity and cell motility (125,126).

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