REVIEWS

A possible unifying principle for mechanosensation

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Of Aristotle's five senses, we know that sight, smell and much of taste are initiated by ligands binding to G-protein-coupled receptors; however, the mechanical sensations of touch and hearing remain without a clear understanding of their molecular basis. Recently, the relevant force-transducing molecules—the mechanosensitive ion channels—have been identified. Such channel proteins purified from bacteria sense forces from the lipid bilayer in the absence of other proteins. Recent evidence has shown that lipids are also intimately involved in opening and closing the mechanosensitive channels of fungal, plant and animal species.

All creatures have mechanical senses: insects hear, and, when touched, worms twitch and sea anemones contract. Touching the front of a unicellular paramecium makes it swim backward; touching its posterior makes it spurt forward. Plant roots and shoots respond to gravity (gravitropism), and stems proportion the growth in their height, versus the growth in their girth, based on the amount of jostling by wind and rain (thigmomorphogenesis). Besides the ear and the skin, animals have many other mechanosensors; for example, the circumventricular organs (for determining systemic osmolarity), baroreceptors (blood pressure), spindle receptors (muscle stretch), proprioceptors (limb positions) and so on. Our bones measure stress during periods of growth or regeneration, and our tongues sense texture and size so that we don't swallow sand or stones.

Mechanical senses differ from other senses. The molecular bases of sensing odorants, hormones, neurotransmitters and other dissolved ligands (solutes) are well understood: the lock-and-key binding of each ligand to the specific binding pocket of its specific receptor on the plasma membrane. Much less is known, however, about the molecules that sense forces such as osmotic force, thirst, touch, vibration and texture. Many membranes are equipped with mechanosensitive (MS) ion channels that respond to turgor in proportion to the surrounding concentration of water (the solvent). Such channel proteins have been cloned and crystallized from bacteria. Examination of these proteins by genetic, electric, chemical and physical means has found that they are able to directly detect and respond to forces from the lipid bilayer. The study of MS channels in plants and animals lags behind, partly because their anatomical complexities resist reductionistic approaches. Nonetheless, recent findings indicate the involvement of lipids in the gating of MS channels of the worm, the fly, the frog oocyte and in mammals. The same types of channel proteins—for example, transient receptor-potential (TRP) proteins—are now found to sense vibration, touch and osmotic membrane stretch. The possibility is emerging that force detection ultimately occurs at the channel–lipid interface. Displacement at the interface, either by deforming the bilayer or by pulling the channel or the bilayer with a tether, provides the energetic drive for the channel to reach the open conformation.

This Review covers many of the principal advances in this field, and draws on widely diverse research projects: from hair cells to bacteria. Traditional compartmentalization of research disciplines, such as the segregation of micro- and neuro-biology, often hampers dialogue among projects. Yet, life's basic machineries—such as DNA replication, transcription, translation, the Krebs cycle, electron transport, and now ion filtration and voltage gating—are found to be universal, even though they were originally based, in large measures, on experiments done in microbial systems. This Review asks whether there is a common physicochemical basis that determines how channel proteins sense force, which may serve to unite the varied biological manifestations. Even though touch, hearing and osmosis are seemingly disparate fields of research, they all deal with a single physical parameter—force. Regardless of frequency or duration, a dyne is a dyne.

MS channels allow bacteria to withstand rain

Life is largely aqueous chemistry—we went to Mars looking for evidence of water and, by inference, life. Cells comprise ~80% water, and de- or over-hydration can be lethal. Osmotic force, a measure of water content, is therefore fundamental to cell survival. Even though water is 55.6 M, it only requires a 10 milli-osmolar difference across a barrier to produce an osmotic pressure of ~180 mm Hg ($\sim 2.5 \times 10^5$ dyn cm^{-2}), which generates a ~12 dyn cm^{-1} stretch force on the surface of a 2-μm-diameter sphere. Surprisingly, the proteins in bacteria that directly gauge such stretch forces were only discovered in the last decade, and only by serendipity. When a cell is caught in the rain, or following laboratory dilution, the inward diffusion of water through the lipid bilayer—water channels are not needed—generates a huge turgor that can rise to hundreds of atmospheres (10^8 dyn cm^{-2}): far beyond what the cell envelope can withstand. It is known, from work first carried out in the 1950s, that *Escherichia coli* bacteria release their osmolites (solutes) upon an osmotic down-shock (Fig. 1a). For instance, a 1-in-100 aqueous dilution of the culture drains the cellular ^{14}C-labelled proline pool by >95% (ref. 1). Tracing other osmolites, such as K^+, lactose and ATP, gave similar results[2]. However, osmolite-depleted bacteria retain their macromolecules, and begin protein synthesis within minutes upon return to normal medium[1]. The identity of the 'emergency valves' for solute release remained elusive for almost half a century. Though MscL and MscS (mechanosensitive channel of large or small unitary conductance) were postulated to be these valves when their electrical activities were discovered in 1987 (ref. 3), this role was not proved until 1999 when Booth and co-workers[4] showed that Δ*mscL*

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Δ*mscS* double mutants lyse even upon a rather mild down-shock. MscL and MscS have redundant functions and single mutants have no clear phenotype, so they were not discovered in the extensive and intensive genetic dissection of *E. coli* physiology in the last century, but were, in fact, identified in a patch-clamp survey (discussed below). MS channels are just one—albeit the quickest one—of a bacterium's many defences against de- or over-hydration. The slower types of these defences are those that are transcriptionally controlled.

Prokaryotic MS channels

MscL and MscS were encountered in the first electrophysiological survey of bacterial membranes, conducted by Martinac *et al.*[3]. Voltage-controlled (-clamped) patches of *E. coli* membranes produced giant steps in unitary current when the patches were subjected to suction[3], or when the solution bathing the patch was diluted[5]. These MS-channel activities remain following reconstitution into artificial liposomes after membrane dissolution or protein purification[6] (Fig. 1b). The non-selective unitary conductances of MscL (~3 nS) and MscS (~1 nS) are 10–1,000-fold greater than those of the more selective channels commonly studied. Such large signals and unlimited bacterial material allowed the tracing of the channel activity in chromatographic fractions to a single protein, which, in turn, led to the discovery of the *mscL* gene[7,8]. The corresponding small protein has two transmembrane (TM) helices, M1 and M2 (refs 7, 9; Fig. 2, left). Chang *et al.* resolved the crystal structure of the *Mycobacterium tuberculosis* MscL homologue as a homopentamer with the five M1 domains converging to close the pore at the cytoplasmic side[10] (Fig. 2, centre). In 2001, computational modelling and cross-linking experiments led Sukharev *et al.*[11] to a model in which all TM helices recline and rotate, like the iris of a camera, to open a large pore of some 30 Å in diameter (Fig. 2, right). The following year, through observations with electron paramagnetic resonance spectroscopy after site-directed probe attachment, Martinac and co-workers[12,13] concurred with the main premise of this model. The cloning[4] and crystallization[14] of the *E. coli* MscS protein showed that it is a homoheptamer of three-TM subunits with seven converging M3 domains: entirely different from MscL. Genetic, biochemical, biophysical, simulation and other studies on MscL and MscS are periodically reviewed[15–18].

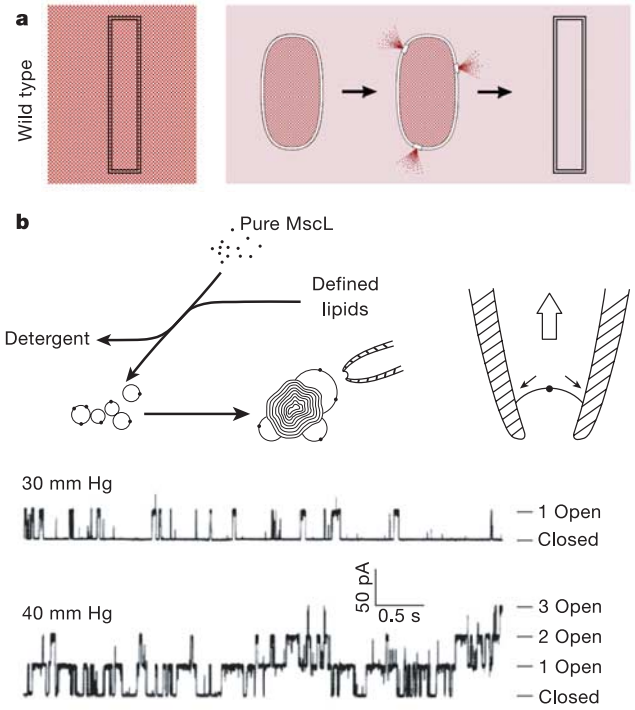

Figure 1 | Bacterial channels function as emergency release valves *in vivo*, and the mechanosensitivity of pure MscL channel protein *in vitro*. a, An *E. coli* cell in a normal environment (left) and in the rain (or upon dilution in the laboratory, right). A bacterium (shown as a rod), having adjusted its cytoplasm to the relatively high osmolarity of the surrounding milieu (shown in dark red, the red dots being solutes, not water), is confronted with a sudden dilution of its environment upon the onset of rain (light red). Entry of water (not shown) through the lipid bilayer swells the bacterium (now oval-shaped) and stretches open the MS channels to jettison solutes (red puffs), enabling it to reach a new equilibrium and escaping osmolysis (and returns to being rod-shaped). **b**, Purified MscL protein is reconstituted into multilamellar liposomes after replacing the detergent with lipids. Induced liposome blisters can be sampled with a patch-clamp pipette. A suction applied to the pipette (broad open arrow) creates tension (small filled arrows) in the membrane patch and activates MscL proteins. The increase in the number of channel openings in a patch (shown as unitary-conductance steps at the marked levels) is evident when the suction applied to such a patch of lipid bilayer increases from 30 mm Hg to 40 mm Hg (4×10^4 dyn cm^{-2} to 5.3×10^4 dyn cm^{-2}) (modified from ref. 15).

Forces from lipids gate prokaryotic MS channels. Exercising ultimate reductionism, mechanosensitivity was found to be intact when pure MscL protein was placed in bilayers of one[12,13] or two[7] defined lipids (Fig. 1b). Here, the stretch force detected by the protein must come from the lipids themselves, since there are no other components that could contribute. MscL follows a Boltzmann distribution where the mechanical energy partitions the MscL protein between its closed and open conformations, with the midpoint tension (50% of the channel being open) at ~12 dyn cm^{-1} (ref. 19)—a sensitivity presumably tuned for its biological role (Fig. 1a). (The threshold tension of MscL is much lower; wild-type MscS (ref. 20) and MscL gain-of-function mutants[21,22] have even lower thresholds.) MscL functions in bilayers made of ordinary lipids with charged or uncharged heads, saturated or unsaturated tails, and in various mixtures. Shortening the length of the fatty acid chain from 20, to 18, to 16 carbons reduces the energy barrier between the closed and the open state, but does not trigger spontaneous channel opening[12,13].

The prevailing model of the behaviour of MscL and MscS addresses the forces within the lipid bilayer itself. Unlike the aqueous solution, the bilayer is highly anisotropic: having very different physical properties at different depths. The free-energy reduction in ordering waters and lipids at the interface is reflected in a large surface tension between the lipids' polar head groups and the nonpolar tails. However, pressures nearby serve to balance this tension, allowing the bilayer to self-assemble into a stable structure. The force profile of pure lipid bilayers has been calculated by Cantor[23,24] (Fig. 3a) and examined with molecular-dynamics simulation[25,26]. (How the protein–lipid interaction may distort the profile at the interface is unclear, though it is being analysed[27].) The amplitudes of these forces are in the order of hundreds of dyne cm^{-1} (ref. 25); much larger than the lytic tension (tens of dyne cm^{-1}) of the bilayer. Any protein embedded in the bilayer is subject to these strong, localized push or pull forces. Altering the force profile by membrane stretch, or by channel or lipid displacement through a tether (discussed below), may make it energetically more favourable for the channel protein to assume a new conformation; for example, the open state (Fig. 3b). Among the molecular-dynamics simulations carried out by several groups, Gullingsrud and Schulten[26] directed forces to residues within the MscL protein at the level of the lipid's glycerol backbone between the head groups and the tails, where tension is maximal (yellow arrows, Fig. 2). Such a simulation can reveal steric clashes or structural disintegration, which should not happen when a channel opens. They traced the positions of the 111,079 atoms of 1 MscL protein, 365 lipids and 22,308 waters, and found that MscL indeed opens on a 10-ns timescale in an iris-like manner, similar to the original model[11–13].

Besides external forces, the composition of the lipid bilayer itself can affect its internal forces. Adding chemically unrelated cationic

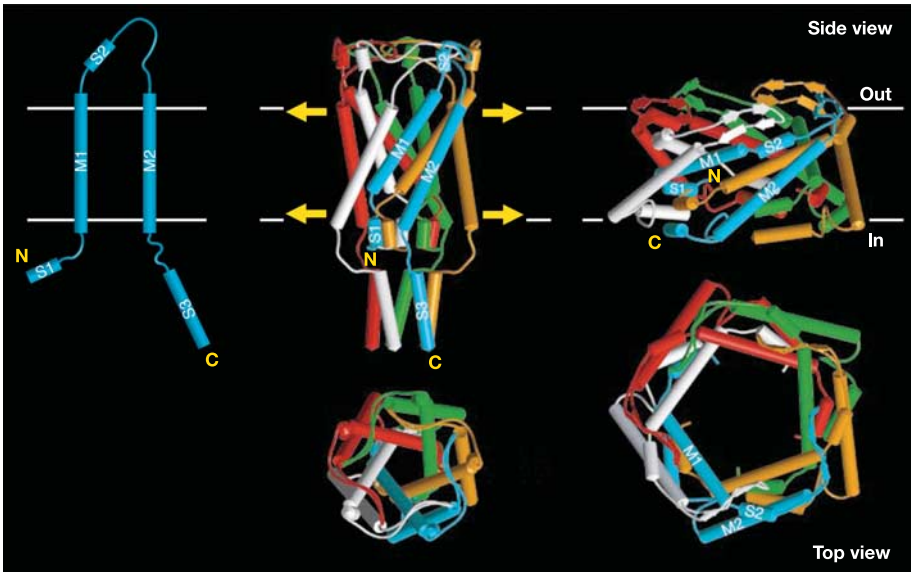

Figure 2 | Opening MscL in *E. coli*. Helical segments (S1, S2, S3) and transmembane helices (M1, M2) in one MscL subunit, as deduced from sequence[7] and other analyses[9] (left). Side (upper centre) and top (lower centre) views of the closed channel backbone structure of the *E. coli* MscL protein, by analogy to the crystal structure of the *M. tuberculosis* MscL homologue[10]. The open structure deduced from both modelling and experimentation[11] (right). Unlike MthK, the prokaryotic K^+ channel that is equipped with a second constriction (the K^+ filter), MscL is like the acetylcholine receptor/channel, in which the open gate doubles as the filter. Here the opening is huge (~30 Å in diameter): befitting its ability to release solutes indiscriminately (as shown in Fig. 1a). The work to increase the area under tension constitutes the free energy difference that partitions the open and closed states. (Modified from ref. 11.)

amphipaths to the membrane of a red blood cell causes it to form cups, while adding anionic amphipaths causes it to form bulges (to crenate)[28]. Regardless of the cause, changes in the bilayer's geometry will distort the force profile contained within it. Indeed, these agents have been shown to reversibly activate MscL and MscS. The potency of the amphipaths to activate such channels is proportional to their lipid solubility[29], and they are effective only when added to one monolayer but not both[13]. The shapes of the added lipids are important, as evident from the behaviour of gramicidin A upon bilayer modifications uncovered by Andersen and co-workers[30]. The usual bilayer-forming phospholipids can be approximated as rods (Fig. 4, shown in red), and the micelle-forming lysophospholipids (with a single fatty-acid chain) as cones (Fig. 4, blue). Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA, a precursor of prostaglandins), can be regarded as inverted cones with smaller heads than tails (Fig. 4, green). Cones, or inverted cones, differentially entered into one monolayer can change the local curvature and the internal force profile, redistributing the tension between the two leaflets (Fig. 3a). Indeed, the addition of lysophosphatidylcholine triggers the opening of MscS (ref. 13). Structurally diverse anaesthetics, which are all lipid soluble, have been theorized to change the bilayer force profile[31]. Indeed, experiments have shown that procaine and tetracaine can activate MscS (ref. 29).

Eukaryotic MS channels

Plants (for example, *Arabidopsis thaliana*) have clear homologues of MscS. Most animal cell membranes present MS conductances, but only a few have been traced to known gene products. Although these differ from MscS and MscL in sequence, some of their properties are quite similar. Patel and co-workers found that the polymodal K^+ channel of mammals, TREK-1 (two-pore domain weak inward-rectifying (TWIK)-related K^+ channel), can be activated by both force and osmolarity. Similar to MscS and MscL, it is also activated by the bulge-forming amphipathic chemicals such as trinitrophenol (called crenaters), but inhibited by the cup-forming amphipaths such as chlorpromazine[32,33]. The cone-shaped lysophosphatidylcholine activates it, and the exaggerated cone lysophosphatidylinositol activates even more effectively[32,34]. Structurally diverse anaesthetics such as chloroform, halothane, isoflurane and diethyl-ether also activate TREK-1 (ref. 35). Negatively charged lipids such as PIP_2 (phosphatidylinositol 4,5-bisphosphate) or phosphatidic acid, when presented to the membrane inner leaflet, also activate TREK-1 (ref. 36), and the charged cone lysophosphatidic acid strongly activates it[37].

MS and some other types of channels are inhibited by the small lanthanide Gd^{3+} (refs 38, 39). The mechanisms of this inhibition are complex and include its interaction with the lipid bilayer[39,40]. Sachs and others found an amphipathic 34 L-amino-acid peptide[41] in a tarantula venom to inhibit the MS conductance in cultured mammalian cells[42]. They also found the synthetic enantiomer of 34 D-amino-acids to be just as effective[43], and concluded that the peptide could not have bound to a channel protein lock-and-key-like, but entered the bilayer to affect the channel's surrounding environment.

Eukaryotic cells have extensive cytoskeletons near the bilayer, which are often assumed to be the transmitters of force. One needs to examine this assumption carefully. Hamill *et al.* examined the MS channels in the complex cell surface of *Xenopus* oocytes[44]. From this surface, they induced blebs that had little or no cytoskeletal elements and continued to encounter MS-channel activities in the bleb membranes[16,44], and it was from such membranes they now traced the channel activities to TRPC1 (transient receptor-potential canonical 1; ref. 45; discussed below). The cortical cytoskeleton network often folds a large excess of membrane bilayer into microvilli or cavaeolae. This network is far more extendable than the bilayer itself, allowing cells to swell without increasing the total bilayer area or tension, which explains why MS currents sometimes cannot be found in whole cells but only in excised patches, where the cytoskeleton is lost[46,47].

Animal sensory cells often have organized microtubules such as the ciliary axoneme (see below). There are also specialized microtubular processes along the length of the long touch-sensing cells in *Caenorhabditis elegans* studied by Chalfie and co-workers[48,49]. The touch-insensitive (loss-of-function) or touch-cell degeneration (gain-of-function)[48–50] worm phenotypes led elegantly to the identification of a series of mechanosensitivity genes (the *mec* genes)

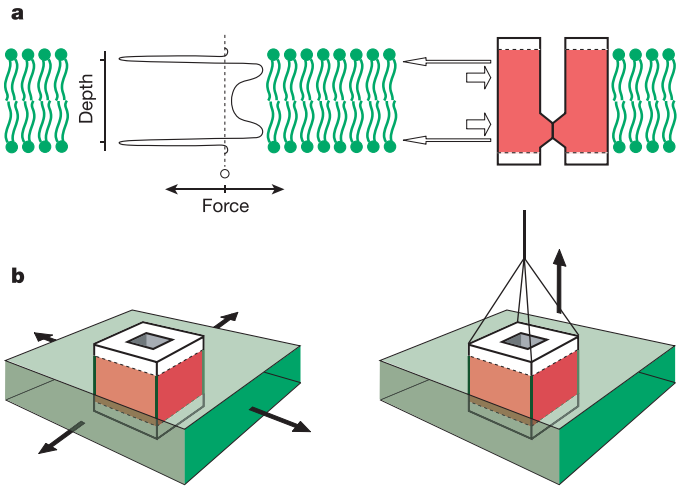

Figure 3 | The intrinsic forces in the lipid bilayer, and how applied forces can open MS channels. a, The intrinsic force profile plotted as its direction and magnitude along the depth of the bilayer (left)[23], and a cartoon of a channel protein in section (right), showing how the sharp tension (narrow arrows) near the lipid necks balanced by more diffused pressure nearby (broad arrows) is exerted on the channel–lipid interface (red). **b**, The forces at the crucial channel–lipid interface (red) will change when the bilayer (green) is stretched or bent (left), or when the channel is displaced from the bilayer through a tether like an elevator (right). It is also possible that the tether, through ancillary proteins, pulls on the lipids surrounding the channel (not shown). In all cases, changes in the force profile at the interface (red) can become the ultimate trigger for the channel's conformational change.

including *mec-4* and *mec-10*. These genes encode channel subunits akin to the epithelial Na^+ channels, and conduct channel currents in frog oocytes[51,52]. The channels, and their associated components, form punctate clusters that are evenly spaced along the worm's microtubular processes. Among the MECs are an extracellular matrix protein (MEC-1) and special tubulins (MEC-7, MEC-12). Previous models described a trans-cellular complex, similar to the original trapdoor model for the vertebrate hair cell (now modified, see below), in which the displacement of the matrix, resisted by the microtubules, gates the channels in between[48,49]. Recent analyses showed that MEC-1, and at least two other gene products in the matrix, are needed to form the punctate clusters. However, removal of the special microtubules through mutation have only limited or no effect on the structure or the function of these clusters[53,54]. These observations, and the fact that the transduction current is reduced but not abolished in *mec-7* mutants[55], have now questioned whether the MS channel is directly gated by tethering to microtubules[53].

Tethering to rigid elements, even if true, does not necessarily imply force transmission. Current cell biology teaches us that meaningful protein–protein contacts, whether ephemeral or long-lasting, are the norm. For example, the signalling machinery of the *Drosophila* photoreceptor, not known for its mechanosensitivity properties, is comprised of rhodopsin, G protein, enzymes and channels, all tied together into a "transducisome"[56] or "signalplex"[57] tethered to an actin cytoskeleton, which serves to deploy and station the complex near the cell surface.

What trips TRP channels?

Several MS channels making recent news belong to the TRP superfamily[45,58,59], the founding member of which was identified in a near-blind *Drosophila* with a transient receptor-potential in its electroretinogram[60]. Forward genetics starting from mechano-insensitive phenotypes led us to these TRP channels without preconceived bias. This has happened six times recently—in the worm[61,62], fly[63,64], mouse[65] and human[66]—and cannot be a coincidence. At least seven homologues of the TRPs identified in this way then became candidates in the ensuing investigations and have now also been tied to mechanosensation.

TRPV4 (transient receptor-potential vanilloid-4; previously called the vanilloid receptor-related osmotically activated channel, or VR-OAC), the most studied mammalian MS TRP channel, is found in many tissues including the circumventricular organs of the central nervous system and inner-ear hair cells. The heterologously expressed whole-cell current through TRPV4 can be activated by cell inflation with pressure[67], by mechanical shear force from bath flow[68], or by mild hypo-osmotic challenges[69,70]. Deleting the three amino-terminal ankyrin repeats does not significantly impair TRPV4's response to hypo-osmolarity[69]. The Bargmann laboratory found the first MS TRPV through *osm-9*-mutant worms, which fail to recoil from an osmolar solution or a nose touch[71]. Worms with normal OSM-9 channels, but deficient in the synthesis of a set of 20-carbon PUFAs (including AA, Fig. 4), showed similar deficits in behaviour that could be restored by a dietary supplement of PUFAs. One such PUFA acts as an irritant that appears to directly activate the channel[72]. The rat TRPV4 is only 24% identical to the worm's OSM-9. Nonetheless, Liedtke *et al.* found that a *trpv4* transgene complements the defects of the *osm-9*-mutant worm, and that the restored behaviours have a threshold and temperature optimum of a warm-blooded animal[73]. Furthermore, a version of TRPV4 deleted of both its amino- and carboxy-terminal cytoplasmic domains, the presumed cytoskeleton-binding sites, remained able to complement the phenotype[73]. The single-channel current of TRPV4 has received far less scrutiny. Unitary conductances of 310 pS (ref. 69), 60 pS (ref. 74), or 30 and 88 pS (ref. 70) have been variously reported from similar experiments. The only report on attempts to activate TRPV4 unitary conductance by direct patch suction was negative[70]. In contrast, a 20 pS MS conductance (in frog Ringer's solution) from oocyte membranes has recently been traced to TRPC1 through liposome reconstitution by Maroto *et al.*[45], who also showed that heterologously expressed human TRPC1 produces MS unitary currents. Although reconstitution of purified TRPC1 has not yet been reported, this work comes closest to showing that certain TRP channels receive their gating force directly from the lipids.

Channels associated with mechanosensation appear in nearly all TRP subfamilies: TRPV, TRPC, TRPA (ankyrin-like), TRPP (polycystin), TRPN (NOMPC, or no mechanoreceptor potential C), TRPY (yeast) and probably TRPML (mucolipins), each having rather different cytoplasmic domains, suggesting that these domains are not

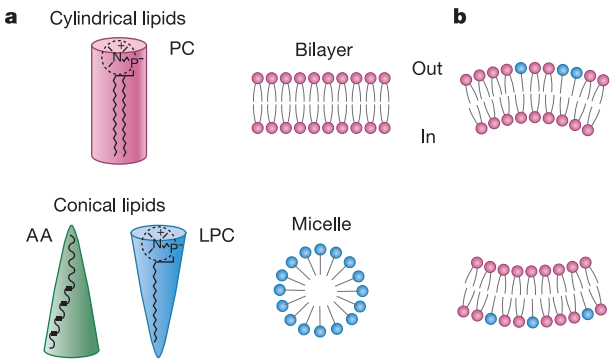

Figure 4 | The shape of bilayer components affects its geometry and intrinsic forces. a, Bilayer-forming phospholipids (shown in red), such as phosphatidylcholine (PC), can be approximated as rods. Micelle-forming lysophospholipids (blue), such as lysophosphatidylcholine (LPC) with only one fatty acid chain, can be regarded as cones. Polyunsaturated fatty acids (green), such as arachidonic acid (AA), approximate inverted cones. **b**, The differential addition of cone-shaped lipids (or other amphipaths, not shown) into one of the monolayers can alter the shape, and therefore the intrinsic forces, of the bilayer. (Modified from ref. 32.)

the crux of mechanosensitivity. TRP channels are polymodal. Heterologously expressed TRPV4, for example, can also be activated by heat, by a phorbol ester, by anandamide and by AA Fig. 4). The well-known heat-sensing vanilloid receptor, TRPV1, identified in the Julius laboratory[75], is also activated by low pH and endogenous inflammatory ligands. The *trpv1* knockout mice and their bladder urothelium have abnormal response to hypo-osmolarity[76]. Though a universal switch for different mode of stimuli may be appealing, there is evidence that different stimuli use different pathways to open TRPV4 (ref. 77).

Strings attached—the roles of tethers

Several TRP channels have now been located within some of the complex MS organs. For example, in the fly chordotonal organ, a matrix material presses against the dendritic cap on top of the sensory cilium, which swells into a dilation about one-third of the way from the tip. Kim, Kernan, and their co-workers found that the only two TRPV channels encoded within the *Drosophila* genome, NAN (encoded by the *Nanchung* gene)[78] and IAV (encoded by the *Inactive* gene)[79], apparently form heteromeric channels that transduce vibrations into receptor potentials. NAN and IAV proteins are found on the cilia but not the rest of the neuron, and it is restricted at and below the ciliary dilation, some distance from the tip (Fig. 5a). How the movement of the cap-cell matrix translates into the forces experienced by the IAV-NAN channel in the ciliary membrane that lies below it is unclear. The NAN (ref. 78) and IAV (ref. 79) proteins have been individually expressed in cultured cells, where they confer hypo-osmotically induced whole-cell current and a rise in cytoplasmic Ca^{2+} levels. A parsimonious interpretation would be that the channel experiences the vibration as a stretch along the membrane plane. Whether there are other proteins that tie them to the axoneme, whether such tethers transmit force, and whether there are roles for other channel subunits (for example, NOMPA, or no mechanoreceptor potential A, located at the distal end of the cilium)[79] await clarification.

The vertebrate hair cell is a clear case in which the gating force is passed on to the MS channel through a tether; though it is debatable whether the tether directly pulls a certain domain from the rest of the channel protein, which is held in place by a resistive force, as originally proposed. Molecular identifications recently enabled great strides in this system. First, TRPN was found in sensory hair cells, and to be required for hearing and balance in zebrafish[80]. Then, cadherin 23 was discovered to be a major component of the tip link[81,82], and judged to be too stiff to comprise the gating spring. Recently, Corey *et al.*[83] showed that *TRPA1* messenger RNA appears at the appropriate time during hair-cell development, and that *TRPA1*-expression knock-down curtails transduction *in vivo*. TRPA1 protein is found at the upper part of the stereocilia, though not just at the very tip. It is also located in the pericuticular zone (perhaps for secretion) and, when present, the entire kinocilium (the true cilium with a microtubular axoneme) (Fig. 5b). With such rapid progress, we are looking forward to the identification of other gene products in this system in the very near future. Meanwhile, fleshing out the biophysical scheme with the molecules so far identified has already led to modifications of the familiar 'trapdoor' model of hair-cell mechano-transduction (Fig. 5c, left). Calculation and simulation show that the long N-terminal ankyrin repeats of TRPA1 are compatible with the elastic property of the gating spring[83,84] (Fig. 5c, right). (The cartoons in Fig. 5c, and similar representations elsewhere, should not be taken literally as the site(s) and the nature of string attachment, in addition to the number and identity of channel subunits and other elements, are unknown. The upper tether can be attached to the gating springs beneath the membrane instead of the channel body.) In the more thoroughly examined multimeric channels, such as MthK (the K^+ channel of *Methanobacterium thermoautotrophicum*; ref. 85), MscL (ref. 12) and MscS (ref. 14), the channel gates open like the iris of a camera. It is not yet clear how the one-dimensional movement of a stereocilium leads to the two-dimensional opening of the TRP tetramers on the side of the cilium, and whether or how the ciliary membrane may move during transduction. TRPA1 is also expressed in neurons of the dorsal root ganglia, the trigeminal nerve, and photoreceptors, making one wonder for which functions the ankyrin repeats serve in these other locations. A role of ankyrin in assembling a TRP tetramer has been suggested[86].

Returning to the main theme of this Review, the vertical movement of a channel by the tip link does not necessarily negate the involvement of the lipid bilayer. It seems possible that such 'elevator'-like movement would still eventually displace the channel with respect to the bilayer's intrinsic force profile (Fig. 3b, right). The mismatch and asymmetry produced by the displacement can, in fact,

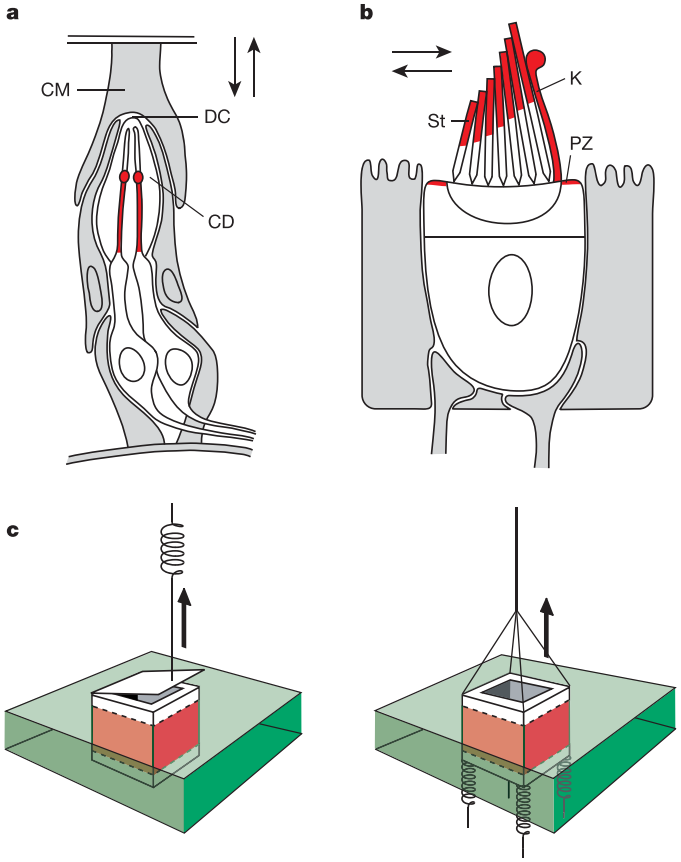

Figure 5 | TRP channels in auditory sensory cells. TRP channels have been located in complex auditory sensory cells, even though the mechanism by which ciliary vibrations (arrow pairs) lead to the iris-like opening of the channels on the side of the cilia is not clear. **a**, The antennal chordotonal organ of *Drosophila*. CM, cap-cell matrix; DC, dendritic cap; CD, ciliary dilation. Red marks the location of NAN (a TRPV-type channel subunit encoded by the *Nanchung* gene). (Redrawn from ref. 78.) **b**, A vertebrate hair cell. St, stereocilia; K, kinocilium; PZ, pericuticular zone. Red marks the location of TRPA1. (Modified from ref. 83.) **c**, Models of the vertebrate hair-cell transduction channel. Molecular identifications have transformed the biophysical trapdoor model (left) to one with a TRPA channel and a stiff cadherin-containing tip link (right). The elastic element of transduction is now assigned to the ankyrin repeats in the four (presumably) TRPA subunits[83,99] (shown as coils), which are presumed to be attached to cytoskeleton and/or myosin (not shown). This current model is compatible with one in which the displacement of the channel protein, with respect to the lipid bilayer, ultimately triggers the channel conformation change as shown in Fig. 3b, right. However, none of these models should be taken literally since we do not yet know the true composition of the transduction channel(s) and how the various channel components contact each other and the lipids. See the main text for some possible variations.

be the final mechano-energetic trigger for the required channel-configuration change. It is even possible that the tether pulls on the rim of an elastic carrel, say, and passes the force through the lipids that surround the channel (Fig. 3b, left), but this possibility has not been investigated. The 'trapdoor' (Fig. 5c, left) is usually interpreted as the separation of individual protein domain(s) from the rest of the channel protein by mechanical work[17]. The 'elevator' entails displacing the entire channel protein from its normal lipid environments, generating tension around the entire circumference, and leading to an iris-like opening (Fig. 3b, right). Either the trapdoor or the elevator model can accommodate the mechanical resistive and elastic elements traditionally described.

It is difficult to imagine TRPA1 being indifferent to the lipids, given its activation by lipid-like compounds. Gillespie and co-workers showed that PIP_2 localizes towards the tip of the hair bundle and is required for both the mechanical transduction current and its adaptations[87]. The mouse TRPA1 was first reported to be a sensor of noxious cold[88]. It is also activated by bradykinin, and the oils of mustard, cinnamon, wintergreen and the like, as well as cannabinoids[89,90].

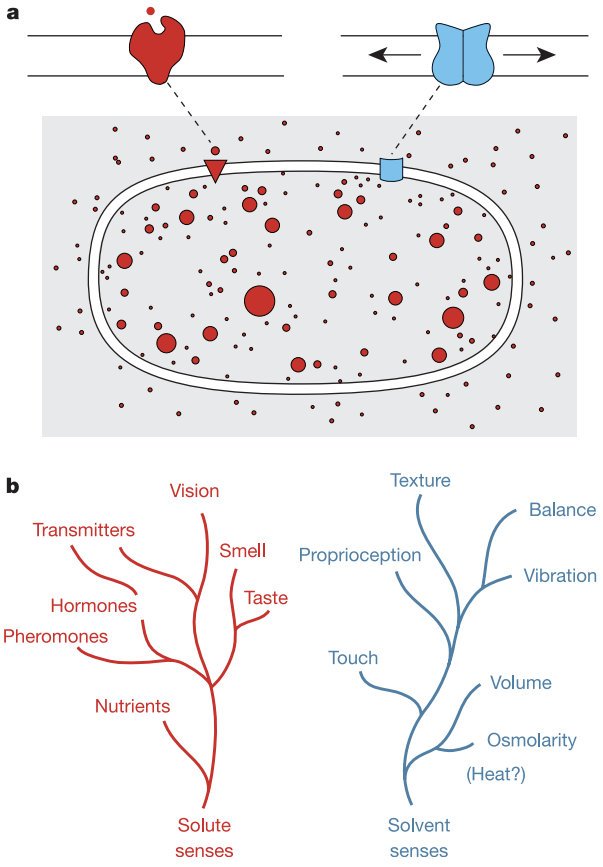

Figure 6 | The disparate sensing of solutes and solvent. a, A diagram of an imaginary early cell equipped with two types of receptors that are required to sense solutes and solvents—the two ingredients of life's chemistry. The dots in the grey background represent water molecules (the solvent) and the red circles represent solutes (molecules dissolved in water). When a cell accumulates solutes, the internal water concentration is reduced and the tendency of water to enter the cell results in a turgor. Both the lock-and-key type of receptors (red) for different solutes (ligands), as well as the turgor sensors (blue) for water (the solvent), are needed for even an early cell to survive. **b**, A hypothetical diagram (not to be mistaken for phylogenetic trees) on the grouping of various senses that emphasizes the discrete separations of the lock-and-key type of sensing of the solutes (red) from the force-from-bilayer type of sensing of the solvent (blue). A further description can be found in the text. (Modified from ref. 100.)

Solute senses versus solvent senses

Specialized sensory cilia develop from embryonic primary cilia, and have evolved from motile cilia similar to the ones still found in *Paramecium*, *Chlamydomonas* and so on. Can we trace the origin of TRP-based mechanosensation beyond motile protists? An MS TRP channel is found in the vacuolar membrane of yeast[91], where it detects osmotic forces *in vitro*[92] and *in vivo*[93,94]. Because all cells have to deal with osmotic force, it may hold a key to the evolutionary origin(s) of mechanosensation.

MscL and MscS proteins are found in most free-living *Bacteria* and *Archaea*. Thus, the principle of mechanical gating by forces from the lipid bilayer most likely evolved before the divergence of these two domains of life some 3.5 billion years ago. It makes teleological sense for these devices to have evolved early on. When early cells separate two solutions, and horde solutes into one, a water gradient—and therefore a turgor—must develop on the chemiosmotic partition. This turgor has apparently been used ever since, as all extant cells today still have to be turgid to be in a growing steady state. This turgidity helps to break the bonds in the network of rigid elements (membrane, cell wall, extracellular matrix, cytoskeleton and so forth) so that new material can be inserted. Off steady states—the sudden large rise in turgor at the onset of rain (over-hydration) and the large fall in turgor in prolonged hot sun (dehydration)—are likely to have exerted selective pressure on early cells to evolve mechanosensors such as the ancestors of TRP, MscS and MscL. Once the basic principle of activation by lipid forces is employed, it seems unlikely that nature would abandon the principle in the detection of other forces later on in evolution, even as newer generations of protein types out-compete the old. As reviewed above, many extant TRP channels in animals still seem to respond to osmotic forces exerted on the lipid bilayer even though their relatives are specialized for hearing, balance, touch, or texture.

Today, we find a myriad of surface receptors for irritants, odorants, hormones, neurotransmitters and growth factors on cell surfaces. They are not sequence homologues, but they could have originated from one or a few receptors by divergent, as well as convergent, evolution. More importantly, the same physicochemical principle of lock-and-key fitting underlies all ligand sensing. Yet one cannot imagine a protein with a lock-and-key water-binding site that discriminates the milli-osmolar differences that cells do, while water exists in tens of molar. Rapid changes in water concentration must therefore be sensed with a different mechanism. From this perspective, it is not difficult to imagine that a parallel myriad of mechanosensors may have evolved from some simple osmotic-force-sensing device in early membranes[95]. Figure 6a shows an 'ur-cell' with the two types of receptors for the various solutes (for example, nutrients and wastes) and for the one solvent (water). Figure 6b shows a schematic representation of how different senses might have evolved throughout the 3.5 billion years of evolution. This is not a molecular dendrogram. The branching pattern has no precise meaning except that the senses were few in number in the beginning, and that sensing of nutrients and water is fundamental, ancient and disparate. This diagram emphasizes the distinctness of the two classes of sensations that originated to deal with solutes versus solvent—the two basic ingredients of life's chemistry.

Complexities and unknowns

The disparity between solute sensing and solvent sensing can help clarify our thinking. Many solute (ligand) receptors do not require a membrane. It is also 'crystal clear' (from X-ray diffraction studies) that some MS channels have no specific ligand-binding sites (for example, MscL, MscS). These examples should not lead us to assert that no receptors employ both principles. Given nature's propensity to tinker opportunistically, it is even likely for some receptor proteins to evolve specific ligand-binding pockets that face the two aqueous compartments, as well as a lipid-facing surface that transduces forces. This complexity may explain certain polymodality[74] or

other differences (for example, the TRPV-dependent behaviour of live worms requires certain features of PUFAs but not necessarily AA[72], while heterologously expressed TRPV4 *in vitro* seems to require an AA metabolite[74]). Complexities can also arise from possible 'signalling lipids' that may act at the protein–lipid–water junction. For example, adding negative charges to the inner leaflet with PIP_2 (ref. 87) will have local electrostatic effects[36,37] as well as secondary effects on lipid distribution and bilayer force profile.

In *On the Soul* (ref. 96), Aristotle argued that there could be no more than five senses, and folded the senses of hot and cold into touch. He might have been on to something. The detection of heat and that of impact seem to convolve in biology. Heat-induced bilayer rearrangement may alter the membrane tension that gates the polymodal TRP channels[97]. Teleologically, have they been designed to sum heat and force (for example, into 'pain')? Mechanistically, can we separate the force sensor from the heat sensor within the same protein? The truism that everything is mechanical, and heat-sensitive, is not helpful. How can a protein–lipid–water complex be designed to be especially sensitive to a rise in thermal agitation? And how can a similar complex become sensitive to its fall? How can some TRPs be constructed to have an unusually high Q_{10} value (in excess of 10)? If the thermosensor is coupled to a voltage sensor[98], what is the coupling mechanism? Doesn't the temperature sensitivity of a channel–lipid complex depend on the entropy of its hydrophobic interactions with water? Why are the temperature mimics (such as capsaicin, menthol and ginger) oily? Why are the agents that numb the sensation amphipathic? Why is the potency of different general anaesthetics proportional to their solubility in olive oil (the Meyer–Overton rule)? Could the protein–lipid–water junction hold even more secrets? Much work lies ahead if the answers to these questions are to be found; perhaps, just like after a long spell of rain, the floodgates of knowledge are about to be opened.

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