# Mechanosensitive ion channels: molecules of mechanotransduction

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## Summary

**Cells respond to a wide variety of mechanical stimuli, ranging from thermal molecular agitation to potentially destructive cell swelling caused by osmotic pressure gradients. The cell membrane presents a major target of the external mechanical forces that act upon a cell, and mechanosensitive (MS) ion channels play a crucial role in the physiology of mechanotransduction. These detect and transduce external mechanical forces into electrical and/or chemical intracellular signals. Recent work has increased our understanding of their gating mechanism, physiological functions and evolutionary origins. In particular, there has been major progress in research on microbial MS channels. Moreover, cloning and sequencing of MS channels from several species has provided insights into their evolution, their physiological functions in prokaryotes and eukaryotes, and their potential roles in the pathology of disease.**

Key words: Mechanosensitivity, Osmoregulation, Ion channels, Bacteria, Archaea, Patch clamp

## Introduction

There are many examples of mechanical force affecting the physiology of living cells. Because mechanical stimuli are everywhere, mechanosensation could represent one of the oldest sensory transduction processes that evolved in living organisms. The idea of mechanically gated (mechanosensitive, MS) ion channels arose originally from studies of specialized mechanosensory neurons (Katz, 1950; Loewenstein, 1959; Detweiler, 1989). As mechano-electrical molecular switches, these convert mechanical force exerted on the cell membrane into electrical or biochemical signals in physiological processes such as cellular turgor control in bacteria and touch and hearing in mammals (García-Añovernos and Corey, 1997; Sachs and Morris, 1998; Hamill and Martinac, 2001; Gillespie and Walker, 2001; Corey, 2003a; Corey, 2003b). Since their discovery in embryonic chick skeletal muscle (Guharay and Sachs, 1984) and frog muscle (Brehm et al., 1984), MS channels have been found in many cell types (Sachs, 1988; Morris, 1990; Martinac, 1993). Significantly, cells that transduce mechanical stimuli into electrical signals are the most common sensory receptors in vertebrates. The variety of information that a vertebrate brain obtains from various mechanoreceptors in the body exceeds that obtained from any other type of sensory receptor cell (Detweiler, 1989).

The patch clamp technique (Hamill et al., 1981) first allowed the direct measurement of single MS channel currents (Hamill, 1983; Guharay and Sachs, 1984; Brehm et al., 1984) and enabled the detection of two basic types of MS channels found in living cells: stretch-activated (SA) and stretch-inactivated (SI) ion channels (Sachs and Morris, 1998). However, at one point, the patch-clamp technique prompted some researchers to interpret MS channel activities as artifacts (Morris and Horn, 1991) because of the way mechanical force is usually applied to cell membranes in such experiments (Gustin et al., 1991; Hamill and Martinac, 2001). Nevertheless, interest in MS channel research was revived following the cloning and characterization of several prokaryotic MS channel proteins (Sukharev et al., 1994; Levina et al., 1999; Chang et al., 1998; Kloda and Martinac, 2001a; Kloda and Martinac, 2001b; Betanzos et al., 2002; Perozo et al., 2002a; Perozo et al., 2002b; Bass et al., 2002) and ion channels implicated in mechanotransduction processes in invertebrates and vertebrates (Tavernarakis and Driscoll, 1997; Colbert et al., 1997; Alvarez de la Rosa et al., 2000; Liedtke et al., 2000; Walker et al., 2000; Di Palma et al., 2002; Kim et al., 2003; Sidi et al., 2003). Because SA channels present a major type of MS channel found in living cells, I discuss the mechanisms that might underlie their gating, together with work that has shaped our understanding of their roles in prokaryotes and eukaryotes.

## Gating of MS channels by mechanical force

MS channels respond to mechanical forces along the plane of the cell membrane (membrane tension), not to hydrostatic pressure perpendicular to it (Gustin et al., 1988; Sokabe and Sachs, 1990; Sokabe et al., 1991). What makes these channels respond to membrane tension is less clear. The answer will not be simple, because not only are MS channels very diverse (Sackin, 1995; García-Añovernos and Corey, 1997; Sachs and Morris, 1998; Hamill and Martinac, 2001), but some voltage- or ligand-gated channels, such as the Shaker-IR $K^+$ channel (Gu et al., 2001), N-type $Ca^{2+}$ channel (Calabrese et al., 2002), NMDA receptor channel (Casado and Asher, 1998) and $Ca^{2+}$-dependent BK channels ($BK_{Ca}$) (Mienville et al., 1996; Kawakubo et al., 1999) also exhibit mechanosensitivity that appears to be irrelevant for their physiological functions.

Two current models describe MS channel gating: the bilayer model and the more speculative, tethered model (Hamill and McBride, 1997). In the bilayer model, first proposed for gating

of MS channels in *Escherichia coli* giant spheroplasts (Martinac et al., 1990), lipid bilayer tension alone is sufficient to gate the MS channels directly, because purified MscL, MscS and other prokaryotic MS channels are still mechanosensitive when reconstituted into liposomes (Sukharev et al., 1994; Häse et al., 1995; Kloda and Martinac, 2001a; Kloda and Martinac, 2001b; Kloda and Martinac, 2001c; Martinac, 2001; Perozo and Rees, 2003). The tethered model invokes direct connections between MS channels and cytoskeletal or extracellular matrix (ECM) proteins, and requires relative displacement of the channel gate with respect to the cytoskeleton or ECM for channel gating (Hamill and McBride, 1997; Gillespie and Walker, 2001). Originally proposed for gating of MS channels in hair cells (Corey and Hudspeth, 1983; Corey, 2003a) and chick skeletal muscle (Guharay and Sachs, 1984), this model might generally apply to MS channels in eukaryotic cells (Hamill and McBride, 1996a; García-Añovernos and Corey, 1997). Whether eukaryotic MS channels respond to deformation of the cytoskeleton and/or the lipid bilayer without a 'tether' is unresolved, although recent evidence suggests that the bilayer model applies to some eukaryotic MS channels (Hamill and Martinac, 2001). Membrane tensions required for half activation of most of the known MS channels are generally around several dynes/cm ($10^{-3}$ N/m) (Sachs, 1988). Such forces can, for example, be produced by differences in transmembrane (TM) osmolarity of a few milliosmols (Martinac, 1993; Sachs, 2004).

## MS channels in prokaryotes

Structural and functional studies of bacterial MS ion channels have enhanced our understanding of mammalian ion channels in ways that electrophysiology and biochemistry alone could not have, thereby acting as a reminder of the usefulness of microbes as model systems. Such bacterial ion channels include: potassium (Schrempf et al., 1995; Doyle et al., 1998; Perozo et al., 1999), glutamate receptor (Chen et al., 1999), sodium (Ren et al., 2001), chloride (Dutzler et al., 2002) and MS ion channels (Martinac et al., 1987; Sukharev et al., 1994; Chang et al., 1998; Sukharev et al., 2001; Perozo et al., 2001; Bass et al., 2002; Betanzos et al., 2002; Perozo et al., 2002a; Perozo et al., 2002b). The patch-clamp technique (Hamill et al., 1981) made possible studies of ion channels in such organisms despite their minute size, which previously limited the earlier two-microelectrode, voltage-clamp electrophysiology studies (Martinac et al., 1994). Prokaryotic MS channel currents have been most extensively studied in *E. coli* (Martinac et al., 1987; Martinac et al., 1992; Zoratti and Ghazi, 1993; Martinac, 2001; Strop et al., 2003), which has three such channels: MscL (large), MscS/MscK (small/kalium, i.e. potassium) and MscM (mini), named for their single-channel conductances of ~3 nS, ~1 nS and ~0.3 nS, respectively (Berrier et al., 1996) (Fig. 1). MS channels have also been detected in numerous other bacteria and the Archaea (Le Dain et al., 1998; Kloda and Martinac, 2002).

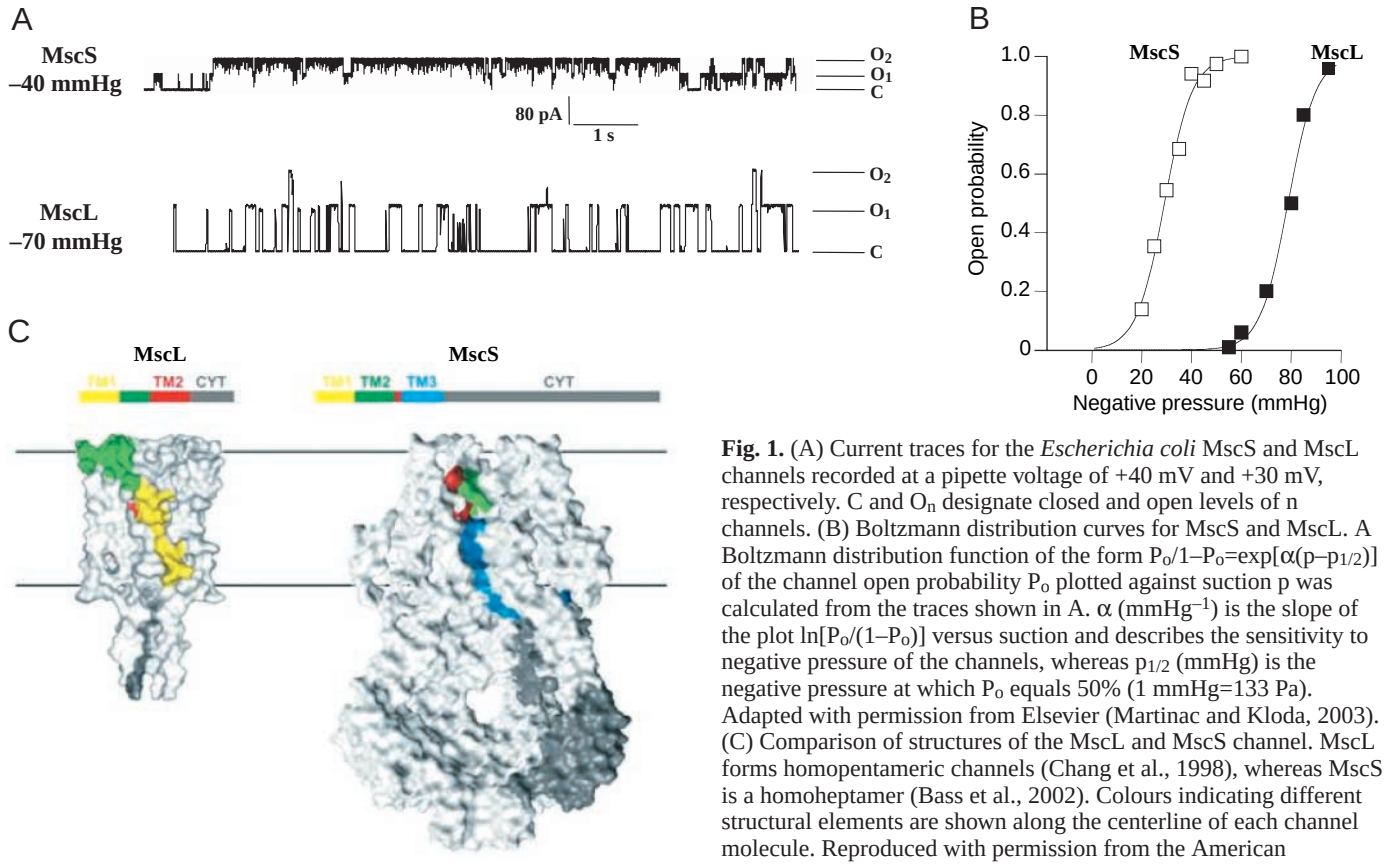


**Fig. 1.** (A) Current traces for the *Escherichia coli* MscS and MscL channels recorded at a pipette voltage of +40 mV and +30 mV, respectively. C and $O_n$ designate closed and open levels of n channels. (B) Boltzmann distribution curves for MscS and MscL. A Boltzmann distribution function of the form $P_o/1–P_o=\exp[\alpha(p–p_{1/2})]$ of the channel open probability $P_o$ plotted against suction p was calculated from the traces shown in A. $\alpha$ ($mmHg^{-1}$) is the slope of the plot $\ln[P_o/(1–P_o)]$ versus suction and describes the sensitivity to negative pressure of the channels, whereas $p_{1/2}$ (mmHg) is the negative pressure at which $P_o$ equals 50% (1 mmHg=133 Pa). Adapted with permission from Elsevier (Martinac and Kloda, 2003). (C) Comparison of structures of the MscL and MscS channel. MscL forms homopentameric channels (Chang et al., 1998), whereas MscS is a homoheptamer (Bass et al., 2002). Colours indicating different structural elements are shown along the centerline of each channel molecule. Reproduced with permission from the American Association for the Advancement of Science (Bezanilla and Perozo, 2002). CYT, cytoplasmic region; TM, transmembrane region.

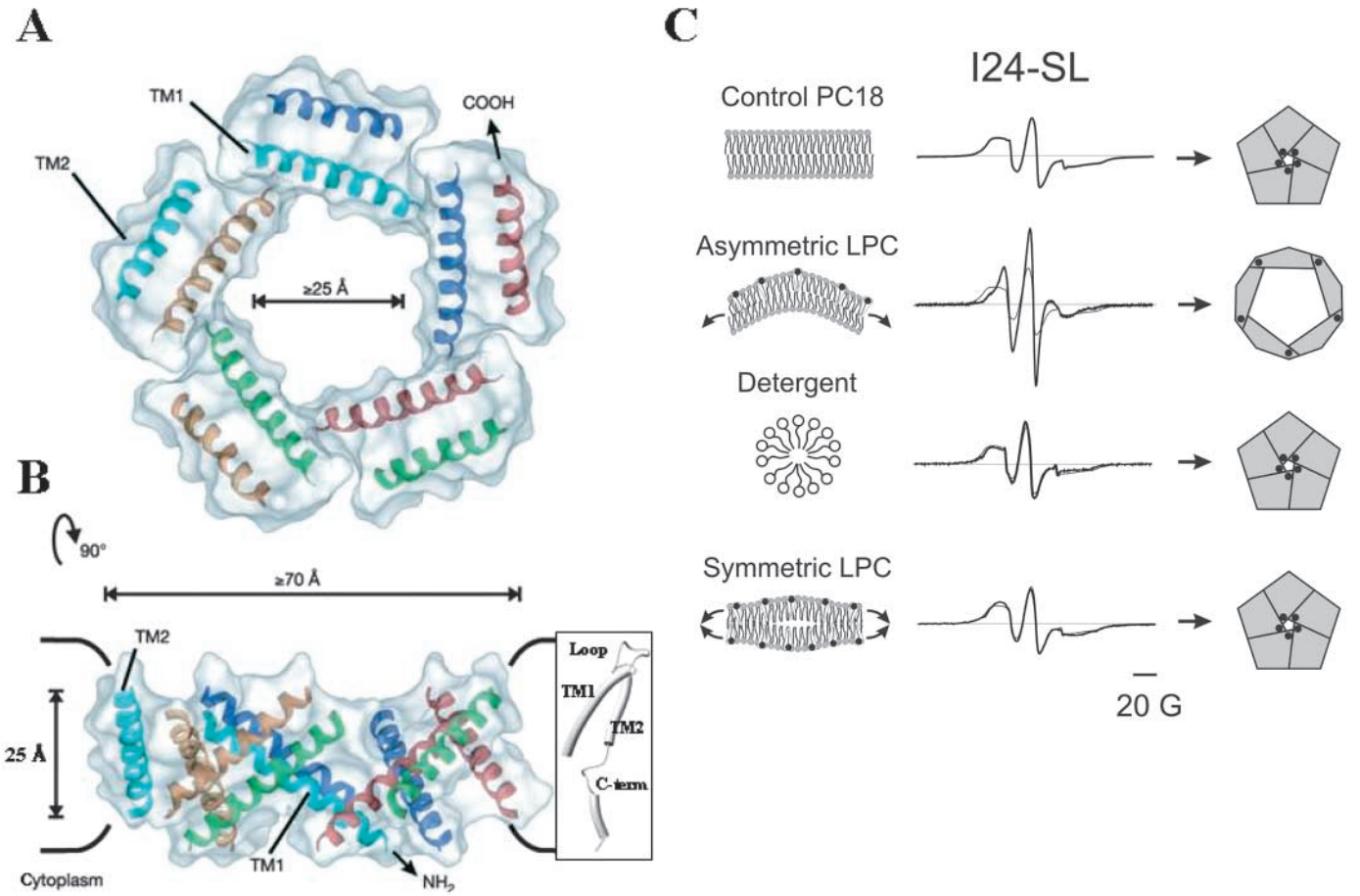


**Fig. 2.** (A) Structure of the MscL channel in the open state viewed from the extracellular side. (B) Side views of the transmembrane domains TM1 and TM2 of the open channel. The inset shows the structure of the channel monomer. Modified with permission from *Nature* (Perozo et al., 2002a). (C) Assymetry of the tension gradient across the lipid bilayer is required for MscL opening. Changes in the spectral line shape from position Ile24 obtained by the EPR spectroscopy were used to monitor the influence of different bilayer tension gradients produced by different lipid environments on the conformation of MscL: symmetric phosphatidylcholine (PC18; closed), asymmetric lysophosphatidylcholine (LPC; open), detergent solution (closed), and symmetric LPC (closed). Note, narrowing of the spectral line after addition of LPC to one monolayer indicates a large increase in spin probe mobility together with complete elimination of any intersubunit spin-spin interaction, which is characteristic of the open channel. Reproduced with permission from *Nature* (Perozo et al., 2002b).

## Bacterial MS channels

MscL, first characterized at the molecular level by Kung and coworkers (Sukharev et al., 1994) comprises 136 residues and shares no significant sequence similarity with known voltage- or ligand-gated ion channels. It has a conductance of ~3 nS and does not exhibit any selectivity for ions (Sukharev et al., 1993). Rees and coworkers (Chang et al., 1998) resolved the three-dimensional (3D) oligomeric structure of the MscL homologue from *Mycobacterium tuberculosis* (Tb-MscL) in the closed state by X-ray crystallography to 3.5 Å and showed that it is a homopentamer whose subunits have two α-helical TM domains, TM1 and TM2, cytoplasmic N- and C-terminal domains and a central periplasmic domain (Fig. 1 and Fig. 2B).

A recent study evaluated two potential triggers of MscL gating by the bilayer mechanism: (1) protein–lipid-bilayer hydrophobic mismatch and (2) membrane curvature (Perozo et al., 2002a,b) (Fig. 2). Perozo et al. examined structural changes in MscL induced by bilayer-deformation forces, combining cysteine-scanning mutagenesis with site-directed spin labelling and electron paramagnetic resonance (EPR) spectroscopy, and analysing channel function by the patch-clamp technique (Perozo et al., 2002b). The open state of MscL has a water-filled pore of >25 Å in diameter that is lined by the TM1 helices from the five subunits (Perozo et al., 2002a) (Fig. 2A), and several studies show that that the channel undergoes a large conformational change when opening and closing (Sukharev et al., 2001; Gullingsrud et al., 2001; Biggin and Sansom, 2001; Betanzos et al., 2002; Colombo et al., 2003; Gullingsrud and Schulten, 2003). The study by Perozo and coworkers (Perozo et al., 2002a) demonstrates that hydrophobic mismatch is not the driving force that triggers MscL opening, although specific mismatch levels could stabilize intermediate states along the kinetic path towards the open state.

The mechanism of mechanotransduction in MS channels is defined by both local and global asymmetries in the transbilayer tension profile at the lipid-protein interface. Addition of lysophosphatidylcholine (LPC) to one monolayer of liposomes reconstituted with MscL channels creates local stresses leading to redistribution of the transbilayer pressure profile in the lipid bilayer. The MS channels could sense these

stresses and open in the absence of externally applied membrane tension. Because MscL opens like the iris of the lens, such that the TM1 helices tilt with respect to the membrane plane and cause the channel to flatten (Betanzos et al., 2002; Perozo et al., 2002a), the reorientation of the TM helices leads to opening of a wide channel pore, which is consistent with the fact that specific hydrophobic mismatch levels stabilize intermediate conformational states of the channel. The effect of LPC on MscL gating appears to be rooted in its insertion into only one monolayer of the liposome membrane, because adding LPC to both monolayers cannot open the MS channel (Fig. 2C).

How to reconcile the apparent tension dependency of MS channels with the two models is best illustrated by the following simple calculation that considers differential expansion of the inner and outer monolayer during patch movements. Assuming that suction applied to a glass pipette deforms the membrane patch from a flat circular disk to a hemisphere with a radius of curvature of 1 μm, then for a membrane thickness of 5 nm, the radii of curvature of the outer and inner monolayers will be 1 and 0.995 μm, respectively. This will result in monolayer areas of 6.28 and 6.22 $\mu m^2$, respectively. If the monolayers are coupled so that they cannot slide past one another, and the number of lipid molecules in each monolayer remains fixed, then this should result in proportional differences in both the area and the thickness of each monolayer. The difference, although only 1%, might be significant considering that the mechanosensitivity of MS channels arises because of differences in protein bilayer mismatch associated with different channel conformations as well as because of the tension difference between the two monolayers (Hamill and Martinac, 2001). Although the mechanism of gating by bilayer-deformation forces applies to both MS channels, the difference in the activation curves of MscL and MscS (Fig. 1B) could result from the difference in the areas occupied by the closed and open structures of the two channels, as well as the difference in size of their TM domains relative to the bilayer thickness.

These key findings regarding the bilayer-controlled functional properties of mechanotransducer channels emphasize that the bilayer is much more than a neutral solvent and actively modulates the specificity and fidelity of signalling by membrane proteins. Elmore and Dougherty investigated lipid composition effects on MscL gating by performing molecular dynamics simulations (Elmore and Dougherty, 2003). They found that protein-lipid interactions are clearly altered by head-group changes, and produce conformational differences in the C-terminal region of MscL. In addition, all their simulations showed evidence of hydrophobic matching between MscL and the lipid membrane, and indicated further that protein-lipid interactions could be more important for proper MscL function and assembly than are protein-protein interactions.

Booth and coworkers have cloned MscS, and its close relative MscK, from *E. coli* (Levina et al., 1999). MscS, encoded at the *yggB* locus, is a small, 286-residue membrane protein. By contrast, MscK, encoded by *kefA*, is a large, multidomain 120 kDa protein comprising 1120 residues. Originally, MscS and MscK were considered to represent a single type of bacterial MS channel because, in patch-clamp experiments, they show similar sensitivities to activation by pressure and exhibit conductances of ~1 nS (Martinac et al., 1987; Sukharev et al., 1993). However, their activities can be distinguished (Li et al., 2002).

The MscS channel is voltage dependent (Martinac et al., 1987; Cui et al., 1995). It is encountered in 100% of protoplast membrane patches characterized by a large number of channels that inactivate rapidly upon sustained application of suction to the patch pipette (Levina et al., 1999). Furthermore, MscS exhibits an anion selectivity ($P_{Cl}/P_K$) of ~1.5-3.0 (Kloda and Martinac, 2002; Sukharev, 2002). MscK was also reported to show some anionic preference (Li et al., 2002). However, mutational analysis has suggested that it might actually be cation specific (McLaggan et al., 2002). MscK activity is found in about 70% of protoplast patches. These are characterized by fewer channels, which do not inactivate upon continuous application of suction. Another distinguishing property is its sensitivity to the extracellular ionic environment (Li et al., 2002). Nonetheless, the overall similarities in conductance, selectivity and sensitivity to membrane tension of MscS and MscK seem to reflect structural similarity between the two channels, as MscK contains an MscS-like domain at its C-terminus (Levina et al., 1999).

The crystal structure of MscS at 3.9 Å resolution (Bass et al., 2002) reveals that the channel folds as a homoheptamer that has a large, cytoplasmic region (Fig. 1C). Each subunit contains three TM domains. The crystal structure indicates that the TM3 helices line the channel pore, whereas the TM1 and TM2 helices constitute the sensors for membrane tension and voltage (Bass et al., 2002). Thus, the MscS crystal structure reveals not only a tension sensor but also a likely voltage sensor. This might provide insight into the structural changes induced by voltage in membrane proteins (Bezanilla and Perozo, 2002).

## MS channels of Archaea

In Archaea, MS channel activities have been documented in *Haloferax volcanii* (Le Dain et al., 1998), *Thermoplasma acidophilum* (Kloda and Martinac, 2001c) and *Methanococcus jannashii* (Kloda and Martinac, 2001a; Kloda and Martinac, 2001b). These channels gate by the bilayer mechanism, have large conductances and low selectivity for ions, are weakly voltage dependent, can be blocked by submillimolar concentrations of gadolinium and are activated by amphipaths (Kloda and Martinac, 2002; Martinac and Kloda, 2003). Note that the lanthanide gadolinium $Gd^{3+}$, which has for some time been considered to be the only common specific blocker of MS channels (Hamill and McBride, 1996b), has a limited use as a tool for MS channel studies because an increasing number of reports indicate that gadolinium does not directly block MS channels, but instead affects the nature of the lipid bilayer and thus indirectly alters the mechanosensitivity of MS channels (Ermakov et al., 2001; Tanaka et al., 2002).

Of particular interest are MscMJ and MscMJLR, the two MS channels of *M. jannashii* (Fig. 3). These have significantly different properties despite sharing 44% sequence identity (Kloda and Martinac, 2001b; Martinac and Kloda, 2003). Both were identified in searches of the *M. jannashii* genome database using TM1 of MscL as a genomic probe. MscMJ, comprising 350 residues, was identified and characterized first (Kloda and Martinac, 2001a); MscMJLR has 361 residues

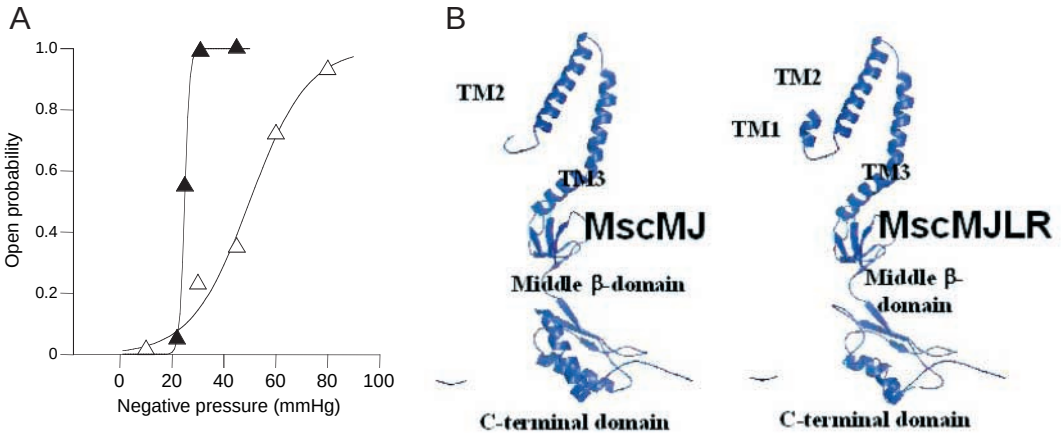


**Fig. 3.** (A) Boltzmann distribution curves for MscMJ (△) and MscMJLR (▲) from *Methanococcus jannashii.* See legend of Fig. 1 for details [adapted with permission from Elsevier (Martinac and Kloda, 2003)]. (B) Structural models of MscMJ and MscMJLR monomers based on the MscS crystal structure, generated by Swiss-Model (Guex and Peitsch, 1997) and viewed by PyMol (PyMOL + AMBER Trajectories, Vanderbilt University Center for Structural Biology; http://structbio.vanderbilt.edu/archives/amber-archive/2002/1207.phtml).

(Kloda and Martinac, 2001b). They exhibit approximately ~28% sequence identity with overlapping residues of the MscS sequence (Kloda and Martinac, 2001b). MscMJ and MscMJLR differ greatly in conductance (~0.3 nS and ~2.0 nS, respectively) (Kloda and Martinac, 2001b). However, they have similar selectivities for cations, $P_K/P_{Cl}$ ~5.0, which resembles the eukaryotic $Ca^{2+}$-permeable SA cation (SA-CAT) channels of skeletal muscle and heart (Hamill and McBride, 1996b). Although strictly not functionally equivalent to MscL and MscS, MscMJ and MscMJLR similarly vary in their sensitivities to pressure: the free energy of activation for MscMJ is ~6 kT (comparable with MscS), and for MscMJLR it is ~18 kT (comparable with MscL). Given that MscMJ and MscMJLR share 44% sequence identity (Kloda and Martinac, 2001b; Martinac and Kloda, 2003), this difference seems to indicate that a structural region might set the level of mechanosensitivity in these channels. The example of the $BK_{Ca}$ channel supports this notion. The ERA sequence present in a stress-axis-regulated exon (STREX) segment of the chick and human relatives of the $BK_{Ca}$ channel is essential for the channel mechanosensitivity. In addition, a single amino acid substitution (A674T) in STREX or deletion of STREX itself in a cloned $BK_{Ca}$ channel from chick completely eliminates the mechanosensitivity of this channel (Naruse et al., 2004) (K. Naruse and M. Sokabe, personal communication).

## Prokaryotic MS channel families

Sequence alignments of MscL and MscS homologues from numerous prokaryotes reveal that they form families that probably have common evolutionary origins. A group of MscL relatives form a separate family of MS channels, encompassing Gram-negative and Gram-positive bacteria (Fig. 4A), as well as representatives from Archaea and fungi (Kumánovics et al., 2003; Martinac and Kloda, 2003; Pivetti et al., 2003). The MscS relatives are more diverse, including several representatives from bacteria, Archaea, fungi and plants (Kloda and Martinac, 2002; Martinac and Kloda, 2003; Pivetti et al., 2003) (Fig. 4B). Because the TM1 domain of MscL was successfully used as a genetic probe for molecular identification of MscMJ and MscMJLR of *M. jannashi*, prokaryotic MS channels might have a common evolutionary ancestry (Kloda and Martinac, 2002). However, this proposal has recently been questioned, given the lack of statistical evidence for a link between the MscL and MscS families, and two groups have proposed that two separate families of MS channels evolved independently (Okada et al., 2002; Pivetti et al., 2003). Nevertheless, sequence similarity between the highly conserved pore-lining helices in the two types of MS channels (i.e. TM1 of MscL and TM3 of MscS) might indicate an evolutionary link between the MscS and MscL families (Kloda and Martinac, 2001a; Pivetti et al., 2003).

## Physiological function of MS channels in prokaryotes

Bacteria possess multiple adaptation mechanisms that enable them to grow in a wide range of external osmolarities (Wood, 1999; Sleator and Hill, 2001). To survive, they require ‘emergency valves’ for release of osmotic stress. Because prokaryotic MS channels have large conductances and mostly lack ionic specificity, they could well serve such a function, acting as osmosensors that regulate the cellular turgor. Indeed, in bacteria, MS channels participate in the response to excessive turgor pressure caused by hypotonic conditions. Without this response, the bacteria lyse. Mutants of *E. coli* lacking both MscL and MscS channel proteins die upon transfer from a medium of high to a medium of low osmolarity (Levina et al., 1999; Booth and Louis, 1999). The third channel, MscM, is insufficient alone to protect them. However, cells lacking only MscS or MscL are fully functional. Obviously, the redundancy provides a safeguard against the deleterious effects of sudden changes in external osmolarity.

MS channels might also sense changes in turgor pressure during cell division and cell growth (Csonka and Epstein, 1996). An increase in turgor stretches the cell envelope and increases cell volume, which might trigger the synthesis and assembly of cell wall components. Indeed, recent evidence suggests that the expression of MscS and MscL is regulated by the stress sigma factor RpoS ($\sigma^S$; RNA polymerase holoenzyme containing $\sigma^S$). RpoS-null mutants are stable

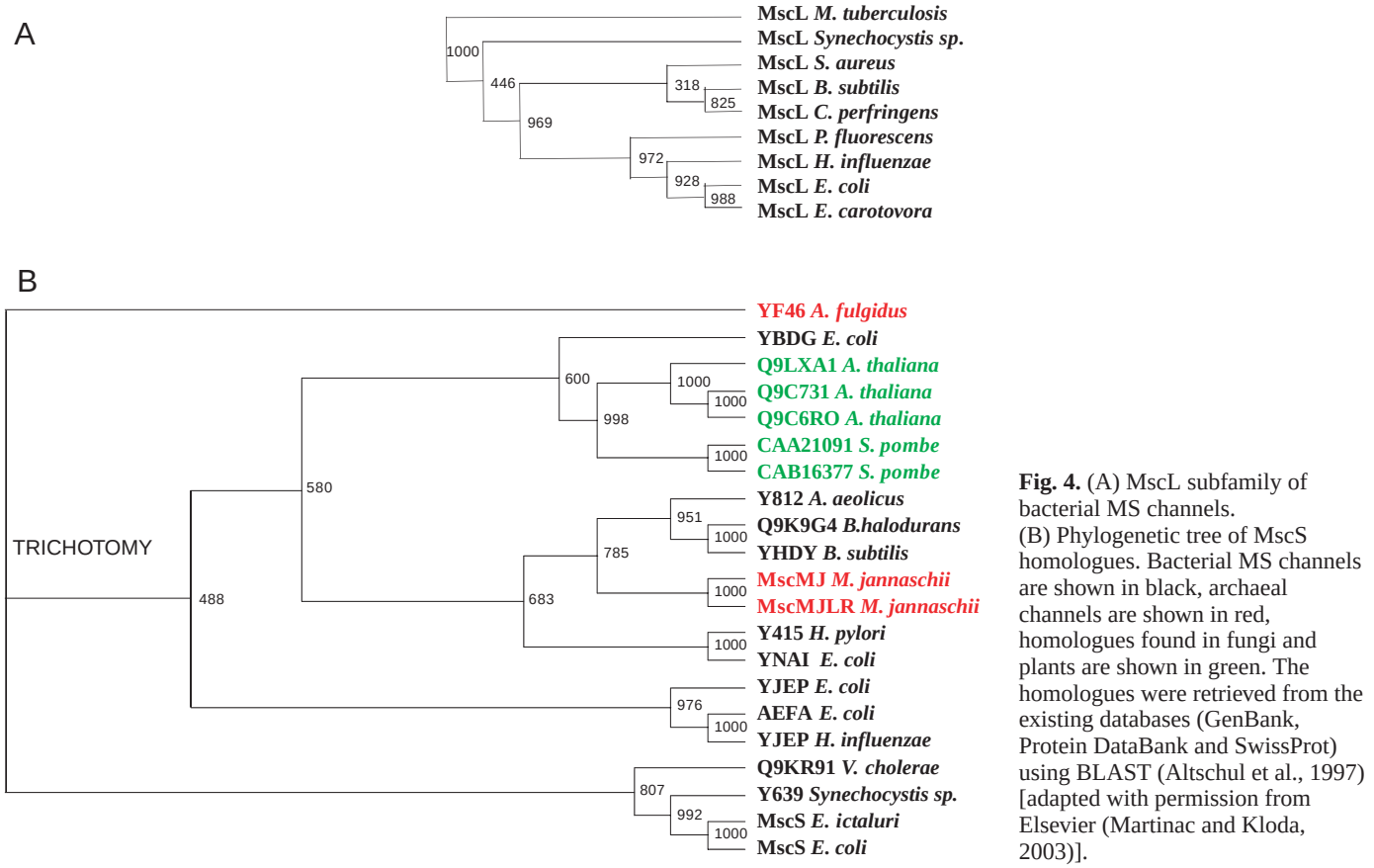


**Fig. 4.** (A) MscL subfamily of bacterial MS channels. (B) Phylogenetic tree of MscS homologues. Bacterial MS channels are shown in black, archaeal channels are shown in red, homologues found in fungi and plants are shown in green. The homologues were retrieved from the existing databases (GenBank, Protein DataBank and SwissProt) using BLAST (Altschul et al., 1997) [adapted with permission from Elsevier (Martinac and Kloda, 2003)].

during exponential growth but are very sensitive to osmotic downshock in stationary phase, which suggests that when the cells undergo cell wall remodelling during entry into stationary phase they need to relieve the turgor pressure. Since the number of MS channels increases in stationary phase, owing to RpoS, their function seems to be linked to cell wall remodelling (Stokes et al., 2003; I. R. Booth, personal communication). The MscK channel must also contribute, since double mutants lacking MscS and MscL are much less sensitive to osmotic downshock than are mutants lacking MscS, MscL and MscK (W. Bartlett and I. R. Booth, personal communication).

The role of MS channels in Archaea has not yet been established, but they probably have functions similar to those of their bacterial counterparts. Expression of archaeal MscMJ in *E. coli* impairs growth of the bacterium, and this can partially be restored in media of high osmolarity (Kloda and Martinac, 2001a). It is possible that the impaired growth of bacterial cells might have resulted from MscMJ being more frequently open in *E. coli* than in *M. jannashii*. The partial rescue of *E. coli* cells in media of higher osmolarity suggests that cellular turgor might be higher in *E. coli* than in the marine *M. jannashii*. Although not much is known about turgor pressure in archaeal cells, turgor is essential for growth and cell wall synthesis in prokaryotes, because the resulting stretching of the cellular envelope is required for its enlargement and consequently for growth of bacterial cells (Csonka and Epstein, 1996). Because floods, drought or volcanic activity can also be expected to occur in the extreme environments Archaea inhabit, archaeal MS channels might also serve as emergency valves for osmoregulation.

## MS channels in eukaryotes

Touch, hearing, proprioception, osmotic gradients, cell swelling, gravitropism and control of cellular turgor are just a few examples of possible MS channel involvement in the physiology of mechanotransduction in eukaryotic cells (Hamill and Martinac, 2001). MS ion channels are found in membranes of a variety of eukaryotic cells. Despite much electrophysiological information about them, molecular characterization and elucidation of their roles in mechanosensory transduction in eukaryotes have been slow in comparison with the progress in our understanding of prokaryotic MS channels. Nonetheless, recent work has identified and electrophysiologically characterized two members of a new family of two-pore-domain (2P-domain), weakly inward-rectifying $K^+$ channels that are mechanosensitive: TREK and TRAAK. Furthermore, mutagenesis studies in *Caenorhabditis elegans*, zebrafish and *Drosophila* have revealed that some of the ion channels belonging to the MEC/DEG (for ‘mechanosensory abnormal/degenerins’) and TRP (for ‘transient receptor potential’) superfamilies might also be mechanosensitive (Hamill and Martinac, 2001; Minke and Cook, 2002). Indeed, genetic work in worms, flies and zebrafish indicates several members of the TRP channel superfamily might play a role in the physiology of mechano- and osmosensation in these organisms (Minke and Cook, 2002;

Corey, 2003b). Although they have yet to be cloned, it is also important to mention the SA-CAT channels, which were first documented in embryonic chick skeletal muscle cells (Guharay and Sachs, 1984), because of the role they might play in muscular dystrophy (Franco-Obregon and Lansman, 1994) and cardiac arrythmias (Hansen et al., 1990; Kohl et al., 2001).

## TREK and TRAAK subfamily of mechano-gated $K^+$ channels

The 2P-domain $K^+$ channels each comprise four TM segments and two pore domains in tandem (Fig. 5). They function as dimers in which both N- and C-termini face the cytosol. The four pore domains of a dimer form the aqueous pore that allows passage of ions through these channels. To date, 15 human family members have been identified. Most of these behave as pure leak or background $K^+$ channels, whose main function is to maintain the resting level of membrane potential. The first to be identified was TWIK-1 (for 'tandem of P domains in a weakly inward-rectifying $K^+$ channel'; (Lesage et al., 1996). TREK-1 (for 'related to TWIK-1') and TRAAK (for 'opened by arachidonic acid') belong to a subfamily (Patel et al., 1998; Maingret et al., 1999a) that gate reversibly in response to membrane stretch. Positive curvature of the membrane (induced by suction applied to the patch pipette) preferentially opens the TREK channels. Stretch activation is also observed in excised membrane patches, indicating that cell integrity is not required for activation by membrane tension.

TREK channels are polymodal $K^+$ channels (i.e. gated by a variety of chemical and physical stimuli) expressed in a variety of tissues, but are particularly abundant in the brain and in the heart (Patel et al., 1999). They are opened by both physical stimuli (e.g. stretch, cell swelling, intracellular acidosis, heat and voltage) (Maingret et al., 1999b; Maingret et al., 2000a; Maingret et al., 2002) and chemical stimuli (e.g. polyunsaturated fatty acids, lysophospholipids, membrane crenators and volatile general anesthetics) (Patel et al., 1998; Maingret et al., 2000b; Patel et al., 1999; Terrenoire et al., 2001). Their activity is downmodulated by phosphorylation of a C-terminal serine residue by cAMP-dependent protein kinase (PKA) (Bockenhauer et al., 2001). The C-terminal domain is essential for mechanosensitivity and acid sensing. Partial deletion of this region impairs activation by membrane tension or lipids (Patel et al., 2001). Protonation of E306 in the C-terminus is responsible for acidic activation, and an E306A substitution locks the channel in an open configuration and prevents the PKA-mediated downmodulation (Honoré et al., 2002). The polymodality of the TREK channels indicates that, in neurons of the central nervous system, they play an important role in physiological (electrogenesis), pathophysiological (ischemia) and pharmacological conditions (anesthesia).

TRAAK is similar to TREK in that it can be activated by arachidonic acid and membrane tension (Maingret et al., 1999a). It is also activated by external but not internal application of the negatively charged amphipath trinitrophenol (TNP), which presumably partitions into and expands the less negative external monolayer to induce a convex curvature (Maingret et al., 1999a). Unlike TREK, TRAAK is not opened by intracellular acidosis in the absence of membrane stretch. Also unlike TREK, it can be activated by the cytoskeletal inhibitors colchicine and cytochalasin, which indicates that the cytoskeleton might constrain tension development in the bilayer (Patel et al., 1998). In common with those of TREK and MscL, the C-terminal domain of TRAAK contains a charged cluster that is crucial for both arachidonic acid activation and mechanosensitivity. TRAAK is widely expressed in the brain, spinal cord, and retina, which indicates that it has a function wider than mechanotransduction in neuronal excitability (Patel et al., 1999).

## ENaC and degenerins

ENaC, the amiloride-sensitive epithelial $Na^+$ channel, is a hetero-oligomer of unknown stoichiometry. In most epithelia, it is composed of α, β and γ subunits, which share 30% sequence identity (Canessa et al., 1994; Schild et al., 1997; Garty and Palmer, 1997). A fourth, δ, subunit is mainly expressed in the testis and ovaries (Darboux et al., 1998). The secondary structure and membrane topology of ENaC are similar to those of bacterial MscL and the ATP-gated P2X receptor channels (North, 1996). Each subunit has two TM domains, TM1 and TM2, intracellular N- and C-termini, and a large extracellular loop. ENaC channels belong to the MEC/DEG superfamily of ion channels, which have diverse functions and include acid-sensing ion channels (ASICs) (Waldmann and Lazdunski, 1998), molluscan FMRFamide-gated channels (Lingueglia et al., 1995), and *Drosophila* $Na^+$ channels expressed in gonads (Adams et al., 1998). The fact that ENaC is a member of the MEC/DEG protein superfamily led to the hypothesis that ENaC is also an MS channel. This is because genetic screens of *C. elegans* have identified several membrane proteins required for touch sensitivity in this nematode. These belong to the superfamily of amiloride-sensitive $Na^+$ channels of the transporting epithelia and degenerins (DEG/ENaC channels), many of which are suspected to be directly gated by mechanical stimuli (Tavernarakis and Driscoll, 1997; Sukharev and Corey, 2004). However, compelling

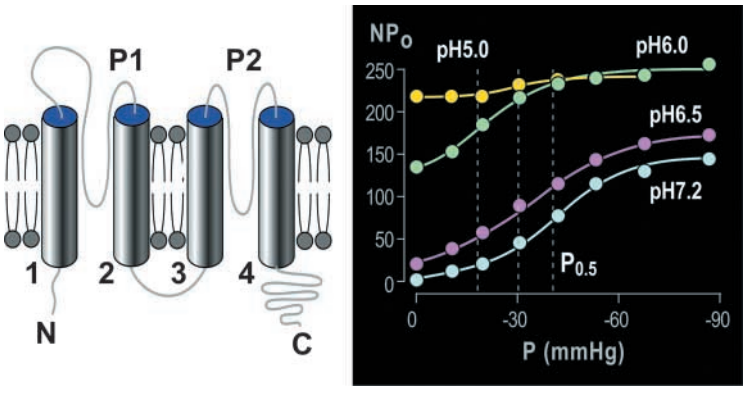


**Fig. 5.** (A) Organization of two-pore-domain (2P-domain) $K^+$ channels. $P_1$ and $P_2$ refer to the pore region in the channel monomer. (B) Activation of TREK by membrane stretch is pH dependent and follows a sigmoidal relationship. $NP_o$ denotes open probability of N number of TREK channels in the patch. For details see text. (Courtesy of E. Honoré.)

electrophysiological evidence for this hypothesis is still lacking (Hamill and Martinac, 2001).

The MEC/DEG subfamily of degenerins is responsible for swelling-induced neuronal degeneration in nematodes. The subfamily includes the MEC-4, MEC-6 and MEC-10 proteins, which are thought to function as subunits of an MS channel that might have a role in touch sensitivity (Driscoll and Chalfie, 1991; Tavernarakis and Driscoll, 1997; Sukharev and Corey, 2004). Although recessive mutations in MEC-4 result in touch insensitivity, dominant mutations in the same gene result in swelling-induced degeneration and lysis of the mechanosensory neurons, which seems consistent with continuously open channels and indicates that specific MEC genes could encode MS channels. Chalfie and colleagues have proposed that MEC-5 in the ECM, and α-tubulin (MEC-12) and β-tubulin (MEC-7) in the cytoskeleton, are tethered to the putative MS channel and this allows transmission of mechanical force directly to the channel (Fig. 6) (Du et al., 1996). Nevertheless, forces transmitted through these proteins might not directly gate the channel but instead produce tension in the lipid bilayer, and thus indirectly activate the MS channels in neuronal membranes (Hamill and Martinac, 2001). To date, there is no direct evidence of MS channel activation in vivo in touch neurons of *C. elegans*, because of the relative inaccessibility of the neurons to patch-clamp recording. However, touch neurons can be patch clamped in vitro as cultured neurons and have recently been shown to exhibit MS channel activity (Strange, 2003). A powerful mix of the patch-clamp electrophysiology and *C. elegans* genetics should significantly advance our understanding of the molecular workings of MEC/DEG-type MS ion channels in mechanosensory neurons.

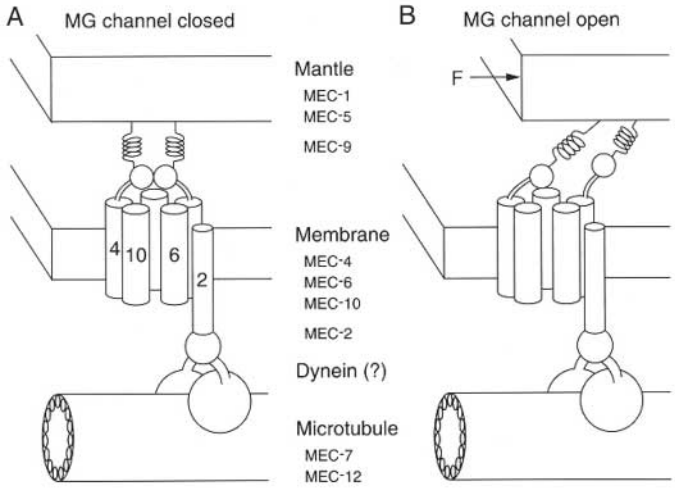


**Fig. 6.** A model of the MS channel from *Caenorhabditis elegans*. (A) The channel is formed from five subunits, including the MEC-4, MEC-6 and MEC-10 membrane proteins, and the integral membrane protein MEC-2, which is homologous to stomatin (a red blood cell protein that binds to the cytoskeleton and regulates cation conductance). In the model, MEC-2 connects the MS channel to microtubules (composed of α- and β-tubulin encoded by *mec-12* and *mec-7*, respectively). (B) The MEC-1 and MEC-5 (a unique collagen) proteins make the mantle, whose pushing by the shearing force F (arrow) results in opening of the MS channel. MEC-9 is an extracellular protein, which does not form the mantle, but is proposed to form the gating spring between the MS channel and the mantle. Figure reproduced with permission from Elsevier (Hamill and McBride, 1996a).

## TRP channels

The >20 TRP-family proteins comprise six subfamilies of cation-selective channels. Each has six TM segments and a membrane topology similar to that of some voltage- and cyclic-nucleotide-gated channels (Harteneck et al., 2000; Montell et al., 2002). The TRP channels are expressed in many tissues in numerous organisms, mediating responses to a variety of physical stimuli (e.g. light, osmolarity, temperature and pH) and chemical stimuli (e.g. odours, pheromones and nerve growth factor) (Minke and Cook, 2002). Several might be inherently mechanosensitive, such as: NompC in the TRPN subfamily (Montell et al., 2002; Montell, 2003); the OSM-9 channel, which is a member of the TRPV subfamily involved in touch and osmosensing in *C. elegans* (Colbert et al., 1997); and PKD-2, which is a member of the TRPP subfamily that mediates mechanosensation in kidney cilia (Nauli et al., 2003).

NompC (for 'no mechanosensory potential') was identified in uncoordinated *Drosophila* mutants that also show an absence or reduction in the mechanoreceptor potentials recorded from external sensory bristles (Walker et al., 2000). It encodes a 1619-residue protein that has 29 ankyrin (ANK) repeats in its N-terminal domain. ANK repeats are responsible for formation of complexes between membrane and CSK proteins, and might couple the NompC channel to cytoskeletal proteins, which could gate or anchor the channel. NompC has not actually been shown to function as an MS channel in patch-clamp experiments. It also remains unclear whether it can function as a homomultimeric MS channel activated by a bilayer-type mechanism or whether it must interact with other proteins. The best evidence that NompC is a transduction channel itself comes from experiments showing that flies carrying a missense mutation between the third and fourth TM domains of NompC show more rapid adaptation to sustained bristle deflections than do wild-type flies (Walker et al., 2000; Sukharev and Corey, 2004).

In contrast to the MEC/DEG proteins, which are expressed only in nonciliated touch cells (Tavernarakis and Driscoll, 1997; Adams et al., 1998), NompC and its nematode homologue Ce-NompC are selectively expressed in ciliated mechanoreceptors (Harteneck et al., 2000). This indicates that the channel could also play a role in hearing. Indeed, *Drosophila nompC* mutants have partially defective auditory responses. However, flies have another MS that is involved in hearing. A member of the TRPV subfamily, named Nanchung, is a $Ca^{2+}$-permeant cation channel localized to the ciliated endings of the *Drosophila* auditory organ (Kim et al., 2003). Deletion of Nanchung causes deafness and a lack of coordination, thus strongly indicating that, although Nanchung might not be the auditory transduction channel itself, it is a component of the auditory mechanosensor (Corey, 2003b).

Recent studies using morpholino oligonucleotides have demonstrated that inhibiting synthesis of the zebrafish orthologue of NompC blocks the 'microphonic' current usually generated when the hair bundle is mechanically deflected, as well as causing behavioural abnormalities characteristic of vestibular dysfunction (Sidi et al., 2003). The *nompC* gene

might thus encode MS channels necessary for auditory hair cell transduction in zebrafish, as in *Drosophila*. A NompC orthologue in mammalian vertebrates has not been identified. The search for the MS channel that is the basis of mammalian and ultimately human auditory mechanotransduction therefore continues (Corey, 2003b).

Two relatives of the vanilloid receptor VR1 [the neuronal membrane receptor for capsaicin and related irritant compounds (Szallasi and Blumberg, 1990; Szallasi and Blumberg, 1999)] – SIC (for 'sic transfected Chinese hamster ovary cells') (Suzuki et al., 1999) and VR-OAC (for 'vanilloid receptor-related osmotically activated ion channel') (Liedtke et al., 2000) – are members of the TRPV subfamily that function as osmotically gated ion channels. SIC is a mammalian $Ca^{2+}$-permeable channel activated by cell shrinkage and inhibited by cell swelling. It is a 563-residue protein whose N-terminal region is shorter than that of VR1. VR-OAC was cloned from *C. elegans*. It is a 871-residue protein that has three ANK repeats in its N-terminal region in common with VR1 (Minke and Cook, 2002). SIC (Suzuki et al., 1999) and VR-OAC (Liedtke et al., 2000) are associated with mechanosensitivity in different animals (Zhou et al., 2003).

PKD2, a member of the TRPP subfamily, functions as a $Ca^{2+}$-permeable ion channel when co-expressed in Chinese hamster ovary cells with PKD1, another much larger (10-12 TM helices) member of the same subfamily (Hanaoka et al., 2000). Localized in the apical cilium of the kidney tubule epithelia, these channels sense fluid flow in ciliated epithelial cells and thus are also probably mechanosensory in nature (Corey, 2003b).

The direct experimental evidence showing that a member of the TRP family is an MS channel comes from a study by Kung and coworkers, who demonstrated that the vacuolar channel of *Saccharomyces cerevisiae*, Yvc1p, exhibits mechanosensitivity in patch-clamp experiments (Zhou et al., 2003). Yvc1p is a Trp-like, $Ca^{2+}$-permeable channel that mediates $Ca^{2+}$ release from the yeast vacuole induced by osmotic upshock. In principle, it is analogous to bacterial MscL and MscS, as a temporary osmotic imbalance is sufficient to activate it. Like MscL and MscS, Yvcp1 also responds to membrane stretch in patch-clamp experiments. This is an important finding, because OSM-9, NompC, PKD-2, VR-OAC and SIC are TRP channels associated with mechanosensation which, like Yvcp1, might all be inherently MS. Further investigation of Yvcp1 could help to elucidate the biophysical principles of how TRP channels detect osmotic pressure and other physical stimuli that affect them.

### MS channels of eukaryotic microbes

In addition to yeast Yvcp1, MS conductances that have known physiological functions also exist in other eukaryotic microbes (Martinac, 1993). An example is the $Ca^{2+}$-selective mechanosensitive conductance of *Paramecium*. This is located in the anterior part of the cell body and underlies the 'avoidance response' that causes the cilia to reverse their direction and increase the frequency of their beating if an obstacle is encountered. A $K^+$-selective conductance in the posterior part of the cell regulates the 'escape response' of the ciliate upon mechanical stimulation of the cell posterior (Machemer and Ogura, 1979; Naitoh, 1984). Another example is the $Ca^{2+}$-permeable MS channels that take part in leaf-topography sensing by the specialized infectious structures in germinating spores of the bean rust fungus *Uromyces appendiculatus* (Zhou et al., 1991) that develop upon contact with the ridges of the lips on bean leaf stomata.

## MS channels in evolution

The finding that MS channels exist in all three domains of living organisms indicates their early evolutionary origins. Very early on, MS channels might have evolved as cellular osmoregulators that measured small changes in the concentration of water across membranes of primordial cells such as bacteria (Sachs, 1988; Kung and Saimi, 1995). These could have later been employed to function in regulation of cell size and volume (Ubl et al., 1988; Christensen, 1987), as well as in more-specialized forms of mechanotransduction, such as gravitropism in plants (Pickard and Ding, 1992) or contractility of the heart (Sigurdson et al., 1992; Kohl and Sachs, 2001; Sachs, 2004). A basic question is whether the bilayer mechanism of gating by mechanical force characteristic of bacterial and archaeal MS channels has been conserved in MS channels of eukaryotic cells. Recent mutational and proteolytic cleavage studies have shown that bacterial MscL can be made as mechanosensitive as any of the eukaryotic MS channels studied in patch-clamp experiments (Yoshimura et al., 1999; Ajouz et al., 2000). Furthermore, experiments in astrocytes and *Xenopus* oocytes indicate that all necessary forces required to activate MS channels in prokaryotic and some eukaryotic cells are transmitted through the lipids. Consequently, the sole function of the cytoskeleton and/or ECM is probably to alter the forces within the lipid bilayer by absorbing mechanical stresses and modifying the time dependence of MS channel adaptation (Hamill and Martinac, 2001).

## MS channels and disease

Abnormalities of MS channel function cause neuronal (Driscoll and Chalfie, 1991; Hong and Driscoll, 1994) and muscular degeneration (Franco and Lansman, 1990), cardiac arrhythmias (Hansen et al., 1990; Franz et al., 1992), hypertension (Kohler et al., 1999) and polycystic kidney disease (Chen et al., 1999). For example, autosomal-dominant polycystic kidney disease (ADPKD), a prevalent genetic disorder that occurs in approximately 0.1-0.2% of the population, might be caused by abnormal $Ca^{2+}$ signalling by polycystins, which act as $Ca^{2+}$-regulated cation channels permeable to $Ca^{2+}$ (Chen et al., 1999) and have been suggested to be mechanosenory in nature (Corey, 2003b).

Note also that atrial fibrillation, the most common cardiac arrhythmia to occur in humans, is associated with passive stretching of the atrial chamber caused by mechanical dysfunction of the heart (Kohl and Sachs, 2001; Sachs, 2004). The stretching causing arrhythmias seems to be dominated by inward cationic currents; hence, the blockade of SA-CAT channels should reduce the extent of the abnormalities in the heart beat. Indeed, the spider venom peptide GsMtx-4, a novel inhibitor of SA-CAT channels, inhibits atrial fibrillation during stretch in the rabbit heart (Bode et al., 2001). There are thus excellent prospects for developing a novel class of drugs targeting SA-CAT channels.

## Conclusion

Our knowledge of the structure and function of MS channels has developed from a serendipitous discovery, to the confusion of artifacts, to molecular identification and structural determination, but is limited compared with what we know about voltage- and ligand-gated channels. The cloning of MscL and MscS, the elucidation of their 3D crystal structures and the demonstration of their physiological role in bacterial osmoregulation are very promising new developments. Similarly, the cloning and genetic analysis of the *mec* genes in *C. elegans*, as well as genetic and functional studies of the TRP-type MS channels, make a significant contribution to our understanding of the role of MS channels in the physiology of mechanosensory transduction. We can expect such substantial developments to continue and to establish MS channels as universal sensory transducers of mechanical force in living cells.

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