10 Mechanosensitive Channels

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Living cells are exposed to a variety of mechanical stimuli acting throughout the biosphere. The range of the stimuli extends from thermal molecular agitation to potentially destructive cell swelling caused by osmotic pressure gradients. Cellular membranes present a major target for these stimuli. To detect mechanical forces acting upon them cell membranes are equipped with mechanosensitive (MS) ion channels. Functioning as molecular mechanoelectrical transducers of mechanical forces into electrical and/or chemical intracellular signals these channels play a critical role in the physiology of mechanotransduction. Studies of prokaryotic MS channels and recent work on MS channels of eukaryotes have significantly increased our understanding of their gating mechanism, physiological functions, and evolutionary origins as well as their role in the pathology of disease.

10.1 Introduction

The idea of mechano-gated or mechanosensitive (MS) ion channels arose originally from studies of specialized mechanosensory neurons (Katz, 1950; Loewenstein, 1959; Detweiler, 1989). By converting mechanical stimuli exerted on the cell membrane into electrical or biochemical signals these channels function in a variety of physiological processes ranging from regulation of cellular turgor and growth in bacteria and fungi to touch, hearing, salt and fluid balance, and blood pressure regulation in mammals (García-Añovernos and Corey, 1997; Sachs and Morris, 1998; Hamill and Martinac, 2001; Gillespie and Walker, 2001; Corey, 2003a,b; Martinac, 2004; Lin and Corey, 2005). After their original discovery in embryonic chick skeletal muscle (Guharay and Sachs, 1984) and frog muscle (Brehm et al., 1984) MS channels have been documented in various types of nonspecialized cells (Sachs, 1988; Morris, 1990; Hamill and Martinac, 2001; Martinac, 2004) as well as in specialized mechanoreceptor neurons (Tavernarakis and Driscoll, 1997; Sukharev and Corey, 2004). Although limited compared to what we know about voltage- and ligand-gated channels our knowledge of the structure and function of MS channels has increased significantly over the last 20 years. The discovery of MS channels in bacteria (Martinac et al., 1987) has ultimately led to molecular identification and structural determination of the MS type of ion channels. The cloning of the bacterial MscL and MscS (Sukharev et al., 1994; Levina et al., 1999), the elucidation of their 3D crystal structures (Chang et al., 1998; Bass et al., 2002) and the demonstration of

their physiological role in bacterial osmoregulation (Booth and Louis, 1999; Levina et al., 1999) have provided basis for intensive research and rapid progress in studies of the structure and function in this class of ion channels (Hamill and Martinac, 2001; Martinac, 2004). Later on, the cloning and genetic analysis of the *mec* genes in *Cenorhabditis elegans* (Tavernarakis and Driscoll, 1997; Goodman et al., 2002; Goodman et al., 2003), genetic and functional studies of the TRP-type MS channels (Montell et al., 2002; Zhou et al., 2003; Corey et al., 2004; Maroto et al., 2005) as well as molecular biological and functional studies of the TREK-1 family of 2P-type potassium channels (Patel et al., 1998, 2001) have further contributed to our understanding of the role of MS channels in the physiology of mechanosensory transduction.

10.2 Evolutionary Origins of MS Channels

MS channels exist in all three domains of living organisms indicating their early evolutionary origins (Fig. 10.1). It is likely that very early on MS channels evolved as cellular osmoregulators designed to measure small changes in the concentration of water across membranes of primordial cells (e.g., bacteria or archaea) (Sachs, 1988; Kung et al., 1990; Martinac, 1993; Kung and Saimi, 1995; Kung, 2005). At some later stage these osmoregulators could have been employed in regulation of cell size and volume (Christensen, 1987; Ubl et al., 1988) or in specialized forms of mechanotransduction, such as gravitropism in plants (Pickard and Ding, 1992), contractility of the heart (Sigurdson et al., 1992; Kohl and Sachs, 2001; Sachs,

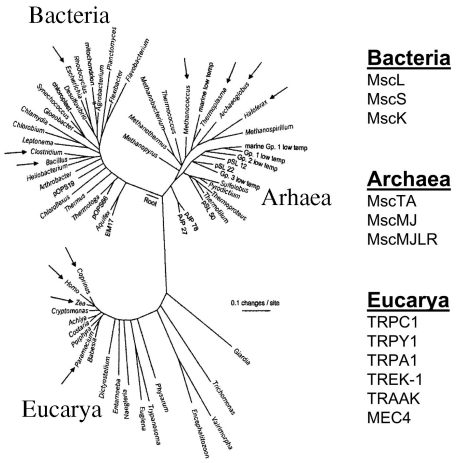

Fig. 10.1 *Ubiquity of MS channels*. Universal phylogenetic tree showing the life on Earth organized in three kingdoms of living organisms based on small subunit tRNA sequences (modified from Pace, 1997; with permission). Organisms, in which MS channels have been identified, are indicated by arrows. MS channels that have been identified at the molecular level are shown on the right.

2004), or hearing (Hamill and Martinac, 2001; Gillespie and Walker, 2001). A basic question that remains to be answered is about the extent to which the mechanism of gating by mechanical force characteristic of prokaryotic (bacterial and archaeal) MS channels has been conserved and adapted to gating of MS channels in eukaryotes.

10.3 Bilayer and Tethered Model of MS Channel Gating by Mechanical Force

MS channels respond to membrane tension caused by mechanical force stretching membranes of living cells (Gustin et al., 1988; Sokabe and Sachs, 1990; Sokabe et al., 1991). Membrane tension required for half activation of most of the known MS channels is usually around several dynes/cm (10^{-3}N/m) (Sachs, 1988). The forces of that magnitude can be produced, for example, by differences in transmembrane (TM) osmolarity of a few milliosmols (Martinac, 1993; Sachs, 2004).

Currently, there are two models which describe gating of MS channels by mechanical force: the bilayer and the more speculative tethered model (Hamill and Martinac, 2001) (Fig. 10.2). According to the bilayer model the tension in the lipid

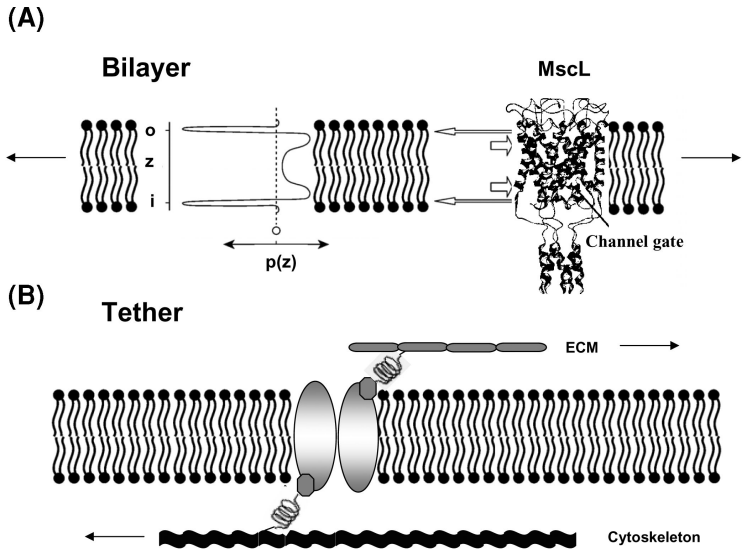

Fig. 10.2 *Bilayer and tethered models of gating MS channels by mechanical force*. (A) The internal pressure profile $p(z)$ plotted along the depth z of the bilayer (left) (Cantor, 1997), and a cartoon of the MscL channel (right). Tension (narrow arrows) pulling on the channel near the lipid head groups is balanced by positive pressure (broad arrows) exerted on the channel–lipid interface. Stretching (black arrows) or bending the bilayer causes a change in the internal pressure profile, which in turn causes change in the channel conformation leading to opening of MS channels, such as MscL (bilayer model). (B) Tension exerted on the cytoskeleton and/or ECM directly gates MS channels without involvement of the lipid bilayer (tethered model).

bilayer alone is sufficient to gate directly the MS channels. Since prokaryotic cells lack a cytoskeleton it is most likely the lipid bilayer is a tension-bearing element transmitting the mechanical force to the MS channels (Martinac, 2004; Kung, 2005). First proposed for the gating of bacterial MS channels (Martinac et al., 1990; Markin and Martinac, 1991), the bilayer model has been documented in various types of MS channels. Recent studies demonstrated that changes in the trans-bilayer tension profile, which gate bacterial MS channels, are caused either by protein–lipid bilayer hydrophobic mismatch and/or membrane curvature (Perozo et al., 2002a,b) (Fig. 10.2). Purified MscL, MscS, and other prokaryotic MS channels remain MS when reconstituted into liposomes (Sukharev et al., 1994; Häse et al., 1995; Kloda and Martinac, 2001a,b,c; Martinac, 2001; Perozo and Rees, 2003) (Fig. 10.3).

A number of eukaryotic MS channels including 2P-type potassium channels TREK-1 and TRAAK (Patel et al., 2001), TRP-type channels TRPC1 (Maroto et al., 2005), TRPY1 (Zhou et al., 2003), TRPY2 and TRPY3 (Zhou et al., 2005) as well as some BK-type potassium channels (Qi et al., 2003, 2005) have also been shown to be gated by bilayer deformation forces. The tethered model invokes direct connections between MS channels and cytoskeletal proteins or extracellular matrix (ECM) 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) (Fig. 10.2). This model was originally proposed for gating of MS channels in hair cells (Corey and Hudspeth, 1983; Corey, 2003a) and chick skeletal muscle (Guharay and Sachs, 1984) and should apply to eukaryotic MS channels in specialized mechanoreceptor cells (Lin and Corey, 2005). A summary of MS channels and their gating mechanism is given in Table 10.1.

The evidence showing that lipids play essential role in opening and closing not only of prokaryotic MS channels but also of the MS channels of fungi, plants, and animals has recently led to a proposal of a possible unifying principle for mechanosensation based on the bilayer mechanism (Kung, 2005). The main idea of the unifying principle is that forces from lipids gate MS channels independently of their evolutionary origin and type of cells in which they are found. According to this principle tethering of MS channels to rigid elements (cytoskeleton or ECM) does not necessarily imply mechanical force transmission. Tethers could serve instead to station the channels close to the cell surface involved in mechanotransduction and to attenuate or amplify the mechanical force and thus adjust the dynamic range within which MS channels operate in a particular cellular setting. Recent mutational and proteolytic cleavage studies have shown that bacterial MscL can be made as MS as any of the eukaryotic MS channels studied in patch-clamp experiments (Yoshimura et al., 1999; Ajouz et al., 2000). Furthermore, experiments in *Xenopus* oocytes seem to indicate that all necessary forces required to activate MS channels are transmitted through the lipids (Zhang et al., 2000). Consequently, the function of the cytoskeleton and/or ECM would consist in altering the forces within the lipid bilayer by absorbing mechanical stresses and modifying the time dependence of MS channel adaptation (Hamill and Martinac, 2001).

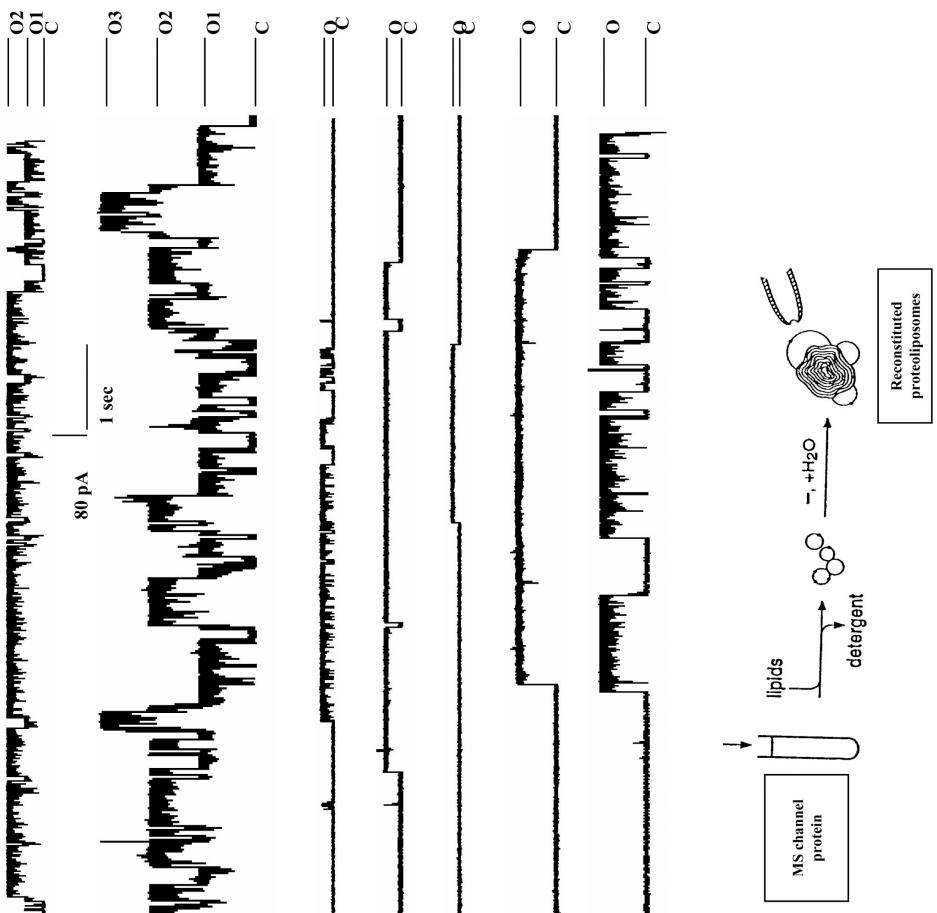

Fig. 10.3 *Current traces of prokaryotic MS channels reconstituted into liposomes.* MS channels found in bacteria and archaea. Current traces of MscL and MscS of *E. coli* are followed by traces of MscA1 and MscA2 of *H. volcanii*, MscMJ and MscMJLR of *M. jannashii* and MscTA of *T. acidophilum*. Currents were recorded at pipette voltage of +40 mV. C denotes the closed state and O_n denotes open state of *n* channels. Note: 1 mm Hg = 133 Pa. *Bottom*: A diagram showing dehydration/rehydration method of MS channel reconstitution into liposomes.

Table 10.1 Summary of cloned MS channels, whose gating mechanism and/or physiological function have been characterized.

MS channel	Source	Gating mechanism	Amphipaths	Physiological function	References
MscL	Bacteria	Bilayer	Yes	Cellular turgor and growth	Häse et al., 1995; Sukharev et al., 1999; Levina et al., 1999
MscS	Bacteria	Bilayer	Yes	Cellular turgor and growth	Martinac et al., 1990; Sukharev et al.,1993 Levina et al., 1999
MscA1	Archaea	Bilayer	NT	Cellular turgor*	Le Dain et al., 1998
MscA2	Archaea	Bilayer	NT	Cellular turgor*	Le Dain et al., 1998
MscMJ	Archaea	Bilayer	Yes	Cellular turgor*	Kloda and Martinac, 2001a
MscMJLR	Archaea	Bilayer	Yes	Cellular turgor*	Kloda and Martinac, 2001b
MscTA	Archaea	Bilayer	Yes	Cellular turgor*	Kloda and Martinac, 2001c
MEC4	*C. elegans*	Tether*	NT	Touch	Tavernarakis and Driscoll, 1997; Hamill and Martinac, 2001
TREK-1	Brain, heart	Bilayer	Yes	Resting membrane potential	Patel et al., 1998, 2001
TRAAK	Brain, spinal chord	Bilayer	Yes	Resting membrane potential	Patel et al., 1998, 2001
EnaC	Rat, human, *C. elegans*	Bilayer/tether*	Yes	Touch	Awayda et al., 2004; Ronan and Gillespie, 2005
TRPC1	*Xenopus* oocytes	Bilayer	NT	Unknown	Maroto et al., 2005
TRPY	Fungi	Bilayer*	NT	Cellular turgor	Zhou et al., 2003, 2005
TRPA1	Hair cells	Tether*	NT	Hearing	Corey et al., 2004; Lin and Corey, 2005
TRPN	Drosophila, zebrafish	Tether*	NT	Touch, hearing	Lin and Corey, 2003; Sidi et al., 2003; Sotomayor et al., 2005
SAKCa	~~Chik~~ heart	Bilayer/tether	Yes	Myogenic tone	Kawakubo et al., 1999; Qi et al., 2005

Note however, that in contrast to the bilayer mechanism there is no single experimental result that provides unequivocal support for the tethered model of MS channel gating (Hamill and Martinac, 2001). Asterisk indicates a likely gating mechanism or physiological function that has not yet been firmly established. NT indicates that the effect of amphipaths has not been tested in the particular type of MS channels.

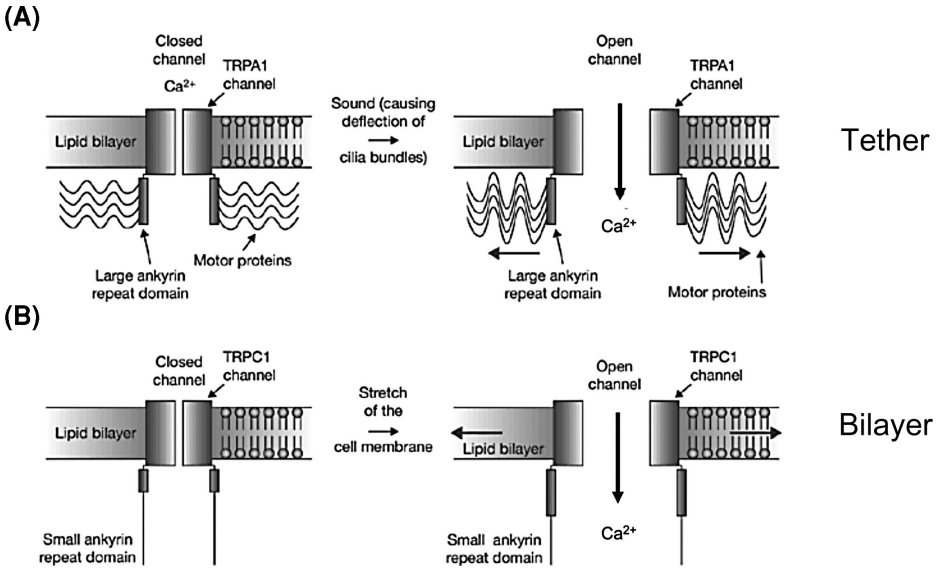

Fig. 10.4 *TRPA1 and TRPC1 acting as mechanosensitive channels*. (A) The functional TRPA1 is probably a homotetramer made of TRPA1 monomers or a heterotetramer composed of TRPA1 monomers and subunits of another channel. Each TRPA1 monomer has 17 ankyrin repeats at the cytoplasmic N-terminal end. It is proposed that the ankyrin repeats function as a spring gating the channel (tethered model). (B) The functional TRPC1 is probably also a homotetramer composed of TRPC1 monomers or a heterotetramer made of TRPC1 monomers and subunits of another channel protein. Each TRPC1 monomer contains three or four ankyrin repeats at the N-terminal cytoplasmic end, which may not be directly involved in channel gating. It is proposed that TRPC1 like MscL, is directly gated by bilayer tension (bilayer model) (modified from Barritt and Rychkov, 2005; with permission).

Of particular interest in regard to the preservation and adaptation of the bilayer mechanism to the gating of eukaryotic MS channels is the TRP superfamily of ion channels that have also been associated with mechanosensation (Fig. 10.4). Among these channels the TRPC1 comes closest to showing that certain TRP channels are gated by mechanical force directly from lipids (Maroto et al., 2005). In contrast, the role of tethers seems to be well established for the hair-cell transduction channel identified to be most likely the TRPA1 protein (Corey et al., 2004). Although the familiar "trapdoor" model of hair-cell mechanotransduction had to be modified the long N-terminal ankyrin repeats of TRPA1 are compatible with the elastic properties of the gating spring (Howard and Bechstedt, 2004; Lin and Corey, 2005; Sotomayor et al., 2005). Nevertheless, it cannot be excluded that TRPA1 may also "sense" the force from lipids given its activation by lipid-like compounds such as mustard oils, cannabinoids or bradykinin (Bandell et al., 2004; Jordt et al., 2004) and requirement of PIP_2 for hair-cell mechanotransduction (Hirono et al., 2004).

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10.4 MS Channels of Bacteria and Archaea

The advent of the patch-clamp technique (Hamill et al., 1981) made possible studies of ion channels in bacteria despite their minute size (Martinac et al., 1994). Bacterial MS channels have been discovered and extensively studied in giant spheroplasts of *E. coli* (Martinac et al., 1987, 1992; Zoratti and Ghazi, 1993; Martinac, 2001; Stokes et al., 2003; Martinac, 2004), which harbors three types of MS channels in its cellular membrane: MscL (*L*arge), MscS/MscK (*S*mall/*K*alium, i.e., potassium), and MscM (*M*ini). The channels are named according to their single-channel conductance, which is ∼3 nS for MscL, ∼1 nS for MscS and MscK, and ∼0.3 nS for MscM (Berrier et al., 1996). MS channels of either MscL- or MscS-type have also been found in other Gram-negative and Gram-positive bacteria as well as in archaea (Le Dain et al., 1998; Kloda and Martinac, 2002; Martinac and Kloda, 2003).

10.4.1 MscL

MscL, cloned and sequenced by Kung and co-workers (Sukharev et al., 1993, 1994), was the first among the MS-type of ion channels characterized at the molecular level. It comprises 136 residues and shares no significant sequence similarity with known voltage- or ligand-gated ion channels. A few years after its cloning Rees and co-workers (Chang et al., 1998) resolved the 3D oligomeric structure of the MscL from *Mycobacterium tuberculosis* (Tb-MscL) by X-ray crystallography. The structure of the channel resolved at 3.5 Å was obtained in the closed state and showed that the channel 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. The structure of the open MscL channel has recently been solved by a combination of cysteine-scanning mutagenesis, site-directed spin labeling, and EPR spectroscopy (Perozo et al., 2002a). The open state of MscL has a water-filled pore of >25 Å in diameter which is lined by the TM1 helices from the five subunits indicating an overall large conformational change in the channel structure during the channel opening (Perozo et al., 2002a). Another recent MscL study employing FRET spectroscopy confirmed these findings by showing a difference of 16 Å in diameter between the closed and the open conformations of the MscL channel (Corry et al., 2005), a result in a close agreement with the EPR spectroscopic study (Perozo et al., 2002a). Several independent studies also showed that the channel undergoes large conformational changes during opening and closing (Cruickshank et al., 1997; Sukharev et al., ~~2000~~; Biggin and Sansom, 2001; Gullingsrud et al., 2001; Betanzos et al., 2002; Colombo et al., 2003; Gullingsrud and Schulten, 2003).

Author: Please include Sukharev et al., 2000 in the reference list.

MscL was also the first MS channel unambiguously shown to be gated by the mechanical force transmitted directly to the channel through the lipid bilayer (Sukharev et al., 1994; Häse et al., 1995) (Fig. 10.2). A recent study evaluated two potential triggers of MscL gating by the bilayer mechanism: (i) protein–lipid bilayer hydrophobic mismatch and (ii) membrane curvature (Perozo et al., 2002b). The

study demonstrated that hydrophobic mismatch is not the driving force that triggers MscL opening, although specific mismatch levels could stabilize intermediate conformational states along the kinetic path toward the open state. According to this study the mechanism of mechanotransduction in MscL and possibly other MS channels is defined by both local and global asymmetries in the trans-bilayer tension profile at the lipid–protein interface. Lipid composition effects on MscL gating were also investigated by performing molecular dynamics simulations (Elmore and Dougherty, 2003). The results of this study showed that protein–lipid interactions were clearly altered by the headgroup changes, leading to conformational differences in the C-terminal region of MscL. In addition, all MD 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 the protein–protein interactions. In accordance with this notion an MscL mutagenesis study showed that the disturbance of the hydrophobic interaction between the membrane lipid and the periplasmic rim of the channel's funnel impaired the function of MscL (Yoshimura et al., 2004). In further support of the significant role that hydrophobic matching could play for the function of MS channels, an independent study using gramicidin A (gA), which exhibits mechanosensitivity in lipid bilayers (Goulian et al., 1998; Hamill and Martinac, 2001), demonstrated that the gA channel could behave as a stretch-activated or stretch-inactivated channel depending on the bilayer thickness (Martinac and Hamill, 2002). These key findings in bilayer-controlled functional properties of mechanotransducer channels emphasize further that bilayer is much more than a neutral solvent by actively modulating the specificity and fidelity of signaling by membrane proteins.

10.4.2 MscS

Among bacterial MS channels MscS was discovered first (Martinac et al., 1987). Besides being activated by membrane tension MscS is also regulated by voltage (Martinac et al., 1987; Cui et al., 1995). It is encountered in 100% of spheroplast 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 $\sim$1.5–3.0 (Kloda and Martinac, 2002; Sukharev, 2002). MscS and its close relative MscK were also cloned in *E. coli* (Levina et al., 1999). MscS, encoded by 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, since in patch-clamp experiments they exhibit similar sensitivities to activation by pressure and have conductance of $\sim$1 nS (Martinac et al., 1987; Sukharev et al., 1993). However, their activities can clearly be distinguished (Li et al., 2002). MscK activity is found in about 70% of spheroplast patches. These are characterized by fewer channels, which do not inactivate upon continuous application of suction. Another distinguishing property of MscK is its sensitivity to the extracellular ionic environment (Li et al., 2002). MscK

was reported to show some anionic preference (Li et al., 2002), although mutational analysis has suggested that it might actually be cation specific (McLaggan et al., 2002). Nevertheless, the overall similarities in their conductance, selectivity, and sensitivity to membrane tension seem to reflect structural similarity between the two channels, since MscK contains an MscS-like domain at its C-terminus (Levina et al., 1999).

MscS was the second MS channel, whose 3D structure was solved by X-ray crystallography (Bass et al., 2002). The crystal structure resolved at 3.9 Å resolution shows that unlike MscL the MscS channel folds as a homoheptamer with each of the seven subunits consisting of three TM domains. The crystal structure also shows 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). The fact that MscS is not only a tension sensor but also a likely voltage sensor might provide insight into the structural changes induced by voltage in membrane proteins (Bezanilla and Perozo, 2002).

10.4.3 Archaeal MS Channels

MS channel activities have also been documented in several archaeal species including *Haloferax volcanii* (Le Dain et al., 1998), *Thermoplasma acidophilum* (Kloda and Martinac, 2001c), and *Methanococcus jannashii* (Kloda and Martinac, 2001a,b). Similar to MscL and MscS these channels are gated by the bilayer mechanism (Fig. 10.3). They all have large conductances and low selectivity for ions. They are weakly voltage dependent, can be activated by amphipaths and are blocked by submillimolar concentrations of gadolinium (Kloda and Martinac, 2002; Martinac and Kloda, 2003). It is important however, to remember that gadolinium (Gd^{3+}) has a limited use as a tool for MS channel studies because an increasing number of reports indicated that Gd^{3+}might not directly block MS channels, but instead should affect the nature of the lipid bilayer and thus indirectly alter the mechanosensitivity of MS channels (Ermakov et al., 2001; Tanaka et al., 2002).

10.4.4 Families of Prokaryotic MS Channels

Sequence alignments of MscL and MscS homologues obtained from a large number of recently cloned genomes of various bacteria and archaea revealed that these channels form families, which have common evolutionary origins. MscL relatives form a separate family of MS channels, encompassing Gram-negative and Gram-positive bacteria, as well as a few representatives from archaea and fungi (Kumánovics et al., 2003; Martinac and Kloda, 2003; Pivetti et al., 2003). In contrast, the MscS relatives are much more diverse and include representatives from bacteria, archaea, fungi, and plants (Kloda and Martinac, 2002; Martinac and Kloda, 2003; Pivetti et al., 2003). It is worth noting that the TM1 domain of MscL was successfully used as a genetic probe for molecular identification of MscMJ and MscMJLR of *M. jannashi*, which belong to the MscS channel family. This is of possible interest

because it seems to suggest that all prokaryotic MS channels might have a common evolutionary ancestry (Kloda and Martinac, 2002). This proposal has however, been questioned, because of the lack of statistical evidence for a link between the MscL and MscS families. Instead it has been proposed that the MscL and MscS families of MS channels evolved independently (Okada et al., 2002; Pivetti et al., 2003). Nonetheless, 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 a possible evolutionary link between MscS and MscL families (Kloda and Martinac, 2001a; Pivetti et al., 2003).

10.5 MS Channels of Eukaryotes

MS ion channels have first been documented in eukaryotes (Brehm et al., 1984; Guharay and Sachs, 1984). Despite their extensive electrophysiological characterization in a large variety of eukaryotic cells (Sachs and Morris, 1998; Hamill and Martinac, 2001), the structural characterization and elucidation of the roles that MS channels play in mechanosensory transduction in eukaryotes have been slow compared to the progress made in understanding of the structure and function of MS channels in prokaryotes. Nonetheless, significant new developments toward understanding of the structure and function of eukaryotic MS ion channels have occurred through recent work on TREK-1 and TRAAK, two MS channels belonging to a new family of two-pore-domain (2P-domain) weakly inward-rectifying K^+ channels, and mutagenesis studies in *Caenorhabditis elegans*, zebrafish and *Drosophila*, which revealed that some of the MEC/DEG (mechanosensory abnormal/degenerins) and TRP (*t*ransient *r*eceptor *p*otential) proteins function as MS channels (Tavernarakis and Driscoll, 1997; Hamill and Martinac, 2001; Patel et al., 2001; Minke and Cook, 2002; Clapham, 2003).

10.5.1 TREK and TRAAK

The 2P domain K^+ channels function as dimers in which both N and C termini face the cytoplasm. To date, 15 human members of this channel superfamily have been identified. Most of the channels behave as pure leak or background K^+ channels, whose main function is to maintain the resting level of membrane potential. Two of them, TREK-1 and TRAAK belong to a subfamily of MS 2P-domain potassium channels (Patel et al., 1998; Maingret et al., 1999a). TREK channels are polymodal (i.e., gated by a variety of chemical and physical stimuli) K^+ channels. They are 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 (stretch, cell swelling, intracellular acidosis, heat, and voltage) (Maingret et al., 1999b, 2000a, 2002) and chemical stimuli (polyunsaturated fatty acids, lysophospholipids, membrane crenators, and volatile general anesthetics) (Patel et al., 1998, 1999; Maingret et al., 2000b; Terrenoire et al., 2001). Characteristic for TREK channels is that

they are preferentially activated by positive curvature of the membrane (induced by suction applied to the patch pipette) similar to MS channels in astrocytes (Bowman et al., 1992; Bowman and Lohr, 1996). In addition, their activity is downmodulated by phosphorylation of a C-terminal serine residue by cAMP-dependent protein kinase (Bockenhauer et al., 2001). The C-terminal domain is essential for mechanosensitivity and acid sensing in these channels. 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 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). Similar to TREK, TRAAK 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). However, 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). It is worth mentioning that stretch activation of both TREK and TRAAK is also observed in excised membrane patches, indicating that cell integrity is not required for activation by membrane tension. In common with TREK and MscL, the C-terminal domain of TRAAK contains a charged cluster that is critical 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).

10.5.2 DEG/ENaC

Genetic screens of *C. elegans* have identified a number of membrane proteins being required for touch sensitivity in this nematode. Four of these proteins, MEC-2, MEC-4, MEC-6, and MEC-10, are candidates for a mechanically gated ion channel complex (Driscoll and Chalfie, 1991; Huang et al., 1995). These proteins belong to a superfamily of amiloride-sensitive Na^+ channels of the transporting epithelia and the degenerins (DEG/ENaC channels) with many of them suspected to be directly gated by mechanical stimuli (Tavernarakis and Driscoll, 1997; Sukharev and Corey, 2004). A recent electrophysiological recording from *C. elegans* touch receptor neurons showed that external mechanical force activated mechanoreceptor currents carried by Na^+ blocked by amiloride thus suggesting that a DEG/ENaC channel is mechanically gated (O'Hagan et al., 2005; Ronan and Gillespie, 2005). ENaC is a hetero-oligomer of unknown stoichiometry composed of α, β, and γ subunits, which share 30%

sequence identity (Canessa et al., 1994; Garty and Palmer, 1997; Schild et al., 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 the bacterial MscL channel 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.

MEC/DEG superfamily of ion channels 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 MEC/DEG subfamily of degenerins are responsible for swelling-induced neuronal degeneration in *C. elegans* and includes the MEC-2, MEC-4, MEC-6, and MEC-10 proteins, which have been shown to underlay mechanoreceptor currents in this nematode (O'Hagan et al., 2005). A role for MEC/DEG proteins in mechanotransduction in *C. elegans* has also been indicated by the finding that mutations in MEC-4 resulted in touch insensitivity and that dominant mutations in the same gene resulted in swelling-induced degeneration and lysis of the mechanosensory neurons, which seems consistent with continuously open channels. Other DEG/ENaC channels including UNC-8 in *C. elegans* (Tavernarakis et al., 1997) and pickpocket in *D. melanogaster* (Ainsley et al., 2003) have also been implicated in mechanosensation.

A hypothetical model of the MEC-mechanotransduction complex proposes that MEC-4 and MEC-10 form the MS transduction channel, which is intracellularly attached via MEC-2 linker to β-tubulin (MEC-7) and α-tubulin (MEC-12) (Du et al., 1996; Ronan and Gillespie, 2005). According to the model the MEC-6 protein should interact with the MEC-4/MEC-10 channel to regulate the activity of the channel, which may also be attached to the ECM. Interestingly, mechanoreceptor currents became reduced but not eliminated in mutants of *C. elegans* affecting MEC-7 β-tubulin found only in touch-receptor neurons (O'Hagan et al., 2005). Therefore, forces transmitted through microtubules and/or ECM proteins might not directly gate the channel but instead produce tension in the lipid bilayer and indirectly activate the MS channels in neuronal membranes (Hamill and Martinac, 2001). In support of this view a recent study provided some evidence for alteration of the ENaC activity by changes in membrane bilayer properties (Awayada et al., 2004). Whatever the mechanism of gating in DEG/ENaC ion channels might be, i.e., bilayer, tether or both, a combination of the patch-clamp electrophysiology and *C. elegans* genetics should significantly advance our understanding of the role of MEC/DEG-type MS ion channels in mechanosensory neurons.

10.5.3 TRPs

Another large and diverse family of ion channels comprises more than 20 TRP (transient receptor potential) proteins organized in six subfamilies of cation-selective channels (Clapham, 2003). Each of the channels has six TM segments and a membrane topology similar to that of some voltage- and cyclic-nucleotide-gated channels

(Hartneck et al., 2000; Montell et al., 2002). Expressed in many tissues in numerous organisms the TRP channels function as cellular sensors mediating responses to a variety of physical (e.g., light, osmolarity, temperature, and pH) and chemical stimuli (e.g., odors, pheromones, and nerve growth factor) (Minke and Cook, 2002; Voets et al., 2005). TRP channels can be activated by sensory stimuli either directly or by a variety of second messengers. They function as specialized biological sensors that are essential in processes such as vision, taste, tactile sensation, and hearing. Several TRP channels may be inherently MS, such as *Drosophila* NompC (now TRPN) identified to have a clear role in mechanosensation (Montell et al., 2002; Montell, 2003; Lin and Corey, 2005), OSM-9 channel, which is a member of the TRPV subfamily involved in touch and osmosensing in *C. elegans* (Colbert et al., 1997), PKD-1 and PKD-2, members of the TRPP subfamily, which mediate mechanosensation in kidney cilia (Nauli et al., 2003), TRPA1 (previously ANKTM1), which is the most likely candidate for the hair-cell mechanotransduction channel (Corey et al., 2004), TRPY1 (formerly Yvc1p), the MS channel found in yeast vacuole (Zhou et al., 2003), and TRPC1 channel, a member of the canonical TRP subfamily, which has been identified as MscCa, the Ca^{2+} permeable MS channel in *Xenopus* oocytes (Maroto et al., 2005).

TRPN (NompC, i.e. *no m*echanosensory *p*otential) 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 is a large protein containing 29 ankyrin (ANK) repeats in its N-terminal domain, which is a unique feature that it shares with TRPA1 implicated in hair-cell function (Corey et al., 2004; Lin and Corey, 2005). ANK repeats may couple the TRPN channel to cytoskeletal proteins, which could gate or anchor the channel. TRPN has however, not been conclusively shown to function as an MS channel in patch-clamp experiments. The best evidence for the TRPN functioning as a mechanotransduction channel comes from experiments showing that flies carrying a missense mutation between the third and fourth TM domains of TRPN showed more rapid adaptation to sustained bristle deflections than the 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), TRPN and its homologue in *C. elegans* are selectively expressed in ciliated mechanoreceptors (Hartneck et al., 2000) suggesting that TRPN could also play a role in hearing given that Drosophila TRPN mutants have partially defective auditory responses (Lin and Corey, 2005). In zebrafish TRPN has been shown to underlie the "microphonic" current usually generated when the hair bundle is mechanically deflected. Inhibiting the synthesis of TRPN by morpholino nucleotides not only inhibited the microphonic current, but also caused behavioral abnormalities characteristic of vestibular dysfunction (Sidi et al., 2003). As in Drosophila TRPN may constitute the MS channel necessary for auditory hair-cell transduction in zebrafish. A TRPN orthologue in mammalian vertebrates has not been identified (Lin and Corey, 2005).

Another MS channel involved in hearing in flies is a member of the TRPV subfamily, named Nanchung. It 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 Nanchung is a component of the auditory mechanosensor (Corey, 2003b). Among the members of the TRPV subfamily two relatives of the vanilloid receptor VR1 (neuronal membrane receptor for capsaicin and related irritant compounds; Szallasi and Blumberg, 1990, 1999), i.e., SIC (for *sic* transfected Chinese hamster ovary cells) (Suzuki et al., 1999) and VR-OAC (for *v*anilloid *r*eceptor-related *o*smotically *a*ctivated ion *c*hannel) (Liedtke et al., 2000), function as osmotically gated ion channels. SIC is a mammalian Ca^{2+}-permeable channel activated by cell shrinkage and inhibited by cell swelling. VR-OAC was cloned from *C. elegans* as a large protein, which like VR1 has three ANK repeats in its N-terminal region (Minke and Cook, 2002).

PKD2, a member of the TRPP subfamily, functions as a Ca^{2+}-permeable ion channel when co-expressed in Chinese hamster ovary (CHO) cells with PKD1, another much larger (10–12 TM helices) member of the same subfamily (Hanaoka et al., 2000). The cytosolic carboxy terminal domain of PKD1 includes a coiled-coil domain which is implicated in protein–protein interactions. The coiled-coil domain of PKD1 interacts with the carboxy terminal domain of PKD2 (Delmas, 2004; Nauli and Zhou, 2004). Localized in the apical cilium of the kidney tubule epithelia, the PKD1/PKD2 complex plays a mechanosensory role in renal epithelia by sensing fluid flow in ciliated epithelial cells (Nauli et al., 2003; Nauli and Zhou, 2004).

Deflection of stereocilia of vertebrate hair cells leads to the opening of MS ion channels at the tips of these cells. Identification of the mechanotransduction channel protein underlying the detection of sound by the ear has been difficult because of the low copy number of a few hundred channels per hair cell. By screening all 33 TRP channels of the mouse using in situ hybridization in the inner ear Corey et al. (2004) have recently provided evidence that the TRPA1 (formerly ANKTM1) constitutes, or is a component of the MS transduction channel of vertebrate hair cells. Similar to other TRP channels TRPA1 is a nonselective cation channel highly permeable to Ca^{2+}. The channel has 17 ankyrin repeats in its N-terminal domain, which led to a proposal that ankyrin repeats are key elements acting as a spring-like gating structure in the activation mechanism of the channel (Fig. 10.4). This proposal has found support in a study examining the elastic properties of the ankyrin repeats by molecular dynamic simulations showing that the extension and stiffness of the large ankyrin-repeat structures matched those predicted by the gating-spring model of the mechanotransduction channel (Sotomayor et al., 2005). Nevertheless, it remains unclear how the channel is activated by the ankyrin spring (Barritt and Rychkov, 2005; Lin and Corey, 2005). Given that TRPA1 is also expressed in neurons of the dorsal root ganglia, the trigeminal nerve and photoreceptors leaving open what the function of the ankyrin repeats in these other locations might be, another proposal has been put forward suggesting that the polyankyrin tether could pass the mechanical force to the channel indirectly through the lipids (Kung, 2005).

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That a member of the TRP family functions as an MS channel was directly demonstrated by the patch-clamp recording from TRPY1, a 300–400 pS channel present in the vacuole of the yeast *Saccharomyces cerevisiae* (Zhou et al., 2003). TRPY1 is a Ca^{2+}-permeable channel that mediates Ca^{2+} release from the yeast vacuole induced by hyperosmotic shock. It is functionally analogous to bacterial MscL and MscS, since a temporary osmotic imbalance is sufficient to activate it. Two distant fungal homologues of TRPY1, TRPY2 from *Kluyveromyces lactis* and TRPY3 from *Candida albicans* have also been shown to function as MS channels in patch-clamp experiments (Zhou et al., 2005). When heterologously expressed in *S. cerevisiae* they retained their mechanosensitivity and could restore the ability to release vacuolar Ca^{2+} upon hypertonic stimulation in the yeast cells having deletion in the *trpy1* gene. Although the conclusive evidence that TRPY channels are gated by bilayer deformation forces is still lacking, because to date they have not been reconstituted and activated in liposomes, it is likely that these channels could respond directly to bilayer tension given their microbial origin and evolutionary distance to TRP channels in higher organisms.

Hamill and colleagues used detergent solubilization of frog oocyte membrane proteins, followed by liposome reconstitution and functional examination by the patch-clamp and identified TRPC1, a member of the canonical TRP subfamily (TRPC1-TRPC7), as MscCa, the verterbrate MS cation channel (Maroto et al., 2005). The evidence came from SDS-PAGE analysis of a membrane protein fraction showing an abundance of 80 kDa protein band that correlated with high MscCa activity in liposomes. The protein band was identified by immunological techniques as TRPC1. Further evidence was provided by injecting a TRPC1-specific antisense RNA in *Xenopus* oocytes, which abolished endogenous MscCa, and heterologous expression of human TRPC1 into CHO-K1 cells, which significantly increased MscCa expression. The role of TRPC1 in mechanosensation seems consistent with the general roles of TRP proteins as sensory detectors (Clapham, 2003).

TRPC1 and TRPA1 present an interesting case of MS channels in terms of their gating by mechanical force. Although definitive evidence for the gating of TRPC1 by the bilayer mechanism is lacking, because the purified TRPC1 protein has not yet been reconstituted and activated in liposomes, the strategy used for its identification as an MS channel, which was similar to that leading to the molecular cloning of MscL (Sukharev et al., 1994, 1997), strongly suggest that these channels could respond directly to bilayer tension (Kung, 2005; Lin and Corey, 2005; Maroto et al., 2005) (Fig. 10.4). In contrast, according to the present evidence TRPA1 is most likely activated directly via the elastic ankyrin spring located in its N-terminal domain (Corey et al., 2004; Lin and Corey, 2005; Sotomayor et al., 2005) (Fig. 10.4). The question is whether the number of ankyrin repeats (17 in TRPA1 and 3 in TRPC1) is what determines how mechanical force is conveyed to a TRP MS channel. The answer to this question would help to resolve and reconcile the current dichotomy between the bilayer and tethered mechanism of MS channel gating.

10.5.4 SAKCa

It is worth considering here mechanosensitivity of the BK_{Ca} channels. These channels have been found in a variety of cells having function in processes, which include firing in neurons, secretion in endocrine and exocrine cells, and myogenic tone in arterial smooth muscle (Gribkoff et al., 2001). Activation of BK_{Ca} by membrane stretch in the absence of calcium has been reported in several types of cells including the heart cells of the chick, which thus led to renaming the channel to SAKCa for stretch-activated Ca-dependent potassium channel (Kawakubo et al., 1999). It has been found that the sequence ERA present in a Stress-axis Regulated Exon (STREX) segment of the chick and human relatives of the SAKCa channels is essential for the channel mechanosensitivity. A single amino acid substitution A674T in STREX or deletion of STREX itself in a cloned SAKCa channel from the chick apparently eliminated the mechanosensitivity of this channel (Tang et al., 2003). A recent study demonstrated that the activity of the SAKCa channels could be modulated by amphipaths chlorpromazine (CPZ) and trinitrophenol (TNP) in a manner strikingly similar to their effect on bacterial MS channels (Qi et al., 2005). These results seem to indicate that the SAKCa channels may respond to the tension in the lipid bilayer. Interestingly, the study reported residual sensitivity to TNP in the STREX deletion mutant channel, but no sensitivity to CPZ. This suggests that the SAKCa channel without STREX may be sensitive to bilayer deformation forces by responding preferentially to tension in one leaflet of the bilayer. An explanation put forward by the study suggested that STREX might interact directly or indirectly via a membrane associated protein, which could sense or transmit the force in the bilayer more efficiently. Consequently, STREX would act as an intermediate structure that could indirectly convey and amplify the mechanical force to the gating mechanism of the SAKCa channels. Future work is expected to resolve the issue of interaction between the bilayer and tether mechanism by focusing on determination of the specific mechanisms of gating eukaryotic MS channels by mechanical force.

10.6 The Role of MS Channels in Cell Physiology and Pathology of Disease

Bacteria possess multiple adaptation mechanisms, which enables them to grow in a wide range of external osmolarities (Wood, 1999; Sleator and Hill, 2001). To release osmotic stress caused by a sudden change in external osmolarity they are equipped with MS channels serving as "emergency valves." It has been well established that bacterial MS channels regulate the cell turgor by opening upon hypo-osmotic shock (Booth and Louis, 1999; Levina et al., 1999) (Fig. 10.5). The channels have large conductances and mostly lack ionic specificity, so that they could well serve such a function. 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. The mini MS channel,

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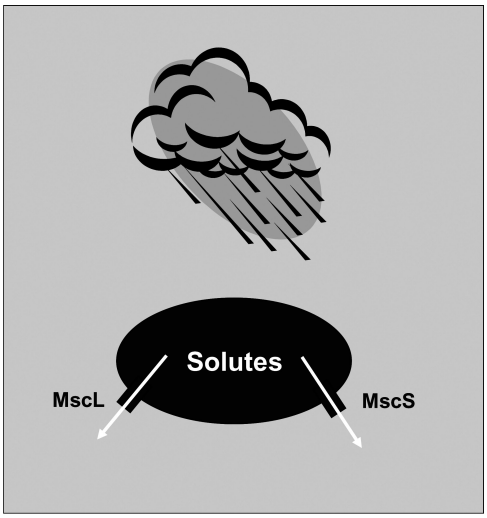

Fig. 10.5 *MS channels in bacteria are essential to maintain cell integrity*. Osmotic stress caused by a sudden drop in external osmolarity (hypo-osmotic shock) opens MscL and MscS in *E. coli* to release excessive turgor pressure. Normally, cell turgor of a bacterial cell is of the order of 4–6 atm. Depending on the magnitude of the hypo-osmotic shock the turgor pressure may increase $\gg$10 atm, which without MS channel opening would cause cell death. $\Delta\Pi$ is osmotic pressure difference in atm (at 22°C), and ΔC is concentration gradient in mol/liter (osmolarity).

MscM, is insufficient alone to protect them. Cells with only MscS or MscL deleted are however, fully functional, which suggest that the redundancy of MS channels provides a safeguard against the deleterious effects of sudden changes in external osmolarity. Another role that MS channels could play in physiology of bacterial cells is in sensing changes in turgor pressure during cell division and cell growth (Csonka and Epstein, 1996). Increase in turgor is required for stretching the cell envelope and increase in cell volume, which may trigger the synthesis and the assembly of cell wall components. This notion has been supported by recent evidence showing that the expression of MscS and MscL is regulated by the stress sigma factor, RpoS (σ^S) (RNA polymerase holoenzyme containing σ^S) (Stokes et al., 2003). During entry into stationary phase the cells undergo cell wall re-modeling, which is accompanied by an increase in the number of MS channels due to RpoS.

The role of MS channels in archaea is most likely very similar to those of their bacterial counterparts. Although not much is known about cellular turgor in archaea, cell turgor is essential for growth and cell wall synthesis in prokaryotes, as stretch of the cellular envelope resulting from turgor is required for enlargement of the envelope and consequently for growth of prokaryotic cells (Csonka and Epstein, 1996). Changes in external osmolarity due to flood, drought, or volcanic activity can also be expected to occur in the extreme habitats of archaea making MS channels

indispensable as emergency valves in cellular osmoregulation in these microbes (Kloda and Martinac, 2001d).

Examples of physiological processes in which MS channels could play a role include touch, hearing, proprioception, osmotic gradients, control of cellular turgor, and gravitropism (Hamill and Martinac, 2001). As demonstrated by genetic studies in worms, flies and zebrafish mechano- and osmosensation in these organisms are regulated by members of the TRP channel superfamily (Minke and Cook, 2002; Corey, 2003b), There is increasing evidence that this ion channel family plays a major role in mechanotransduction in eukaryotic cells (Lin and Corey, 2005). Different types of conductances have been identified that have a physiological function in mechanotransduction in eukaryotic microbes (Martinac, 1993). Examples include the Ca^{2+}-selective and K^{+}-selective MS conductances of Paramecium, which regulate the direction of the ciliary beating during the "avoidance response" and the "escape response" of this ciliate upon mechanical stimulation of the cell anterior and cell posterior, respectively (Machemer and Ogura, 1979; Naitoh, 1984). Neither of the two conductances has to date been characterized at the single channel level.

Abnormalities of MS channel function cause neuronal (Driscoll and Chalfie, 1991; Hong and Driscoll, 1994) and muscular degeneration (Franco and Lansman, 1990; Franco-Obregon and Lansman, 1994), cardiac arrhythmias (Hansen et al., 1990; Franz et al., 1992), hypertension (Kohler et al., 1999), and polycystic kidney disease (Chen et al., 1999). A large number of patients affected by polycystic kidney disease suffer also from serious cardiovascular lesions (Devuyst et al., 2003; Rossetti et al., 2003). Another disease linked to defective MS channels is atrial fibrillation, which is the most common cardiac arrhythmia to occur in humans (Kohl and Sachs, 2001; Sachs, 2004). A role for SA-CAT channels has been implicated in this heart disorder. The blockade of SA-CAT channels by the spider venom peptide GsMtx-4 was shown to reduce the extent of the abnormalities of the heart beat induced by atrial fibrillation in the rabbit heart (Bode et al., 2001). GsMtx-4 blocks MS channels in dystrophic muscle as well (Yeung et al., 2005). Since GsMtx-4 was also found to inhibit TRPC1 channels expressed in the CHO cells (F. Sachs and P. Gottlieb, personal communication), this suggests that SA-CAT channels may be TRPC1 or closely related channels.

10.7 Conclusion

The investigation of MS channels went from a serendipitous discovery to disgrace of artifacts, to be fully re-established over the last decade through molecular identification, structural determination, and functional analysis of a number of MS channels from prokaryotes and eukaryotes. The cloning of MscL and MscS and elucidation of their 3D crystal structure together with the unambiguous demonstration of their physiological role in bacterial osmoregulation have provided a solid basis for further research in this class of ion channels. The cloning and genetic analysis of the *mec* genes in *Cenorhabditis elegans* as well as genetic and functional studies of the TREK

and TRP families of MS channels have greatly contributed to our understanding of the role of MS channels in physiology of mechanosensory transduction. We may expect to see significant future developments in this exciting research field.

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