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MscS-like Mechanosensitive Channels in Plants and Microbes

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ABSTRACT: The challenge of osmotic stress is something all living organisms must face as a result of environmental dynamics. Over the past three decades, innovative research and cooperation across disciplines have irrefutably established that cells utilize mechanically gated ion channels to release osmolytes and prevent cell lysis during hypoosmotic stress. Early electrophysiological analysis of the inner membrane of *Escherichia coli* identified the presence of three distinct mechanosensitive activities. The subsequent discoveries of the genes responsible for two of these activities, the mechanosensitive channels of large (MscL) and small (MscS) conductance, led to the identification of two diverse families of mechanosensitive channels. The latter of these two families, the MscS family, consists of members from bacteria, archaea, fungi, and plants. Genetic and electrophysiological analysis of these family members has provided insight into how organisms use mechanosensitive channels for osmotic regulation in response to changing environmental and developmental circumstances. Furthermore, determining the crystal structure of *E. coli* MscS and several homologues in several conformational states has contributed to our understanding of the gating mechanisms of these channels. Here we summarize our current knowledge of MscS homologues from all three domains of life and address their structure, proposed physiological functions, electrophysiological behaviors, and topological diversity.

INTRODUCTION

Ion Channels. Ion channels are membrane-spanning protein complexes that form a gated macromolecular pore. An open channel can facilitate the diffusion of tens of millions of ions per second from one side of the membrane to the other, down their electrochemical gradient.[1,2] The role played by ions in the excitable membranes of muscle and nerve cells has been studied for more than 100 years,[3] and the importance of ion channels as mediators of the nervous system and their role in human disease are now well-established (several recent reviews include refs 4−6). However, plant and microbial ion channels have also been important subjects of study.[7,8] It is often forgotten that single-cell action potentials were first described in the giant cells of characean algae and that during the 1930s the excitation of squid axons and algal membranes was studied side by side (reviewed in refs 9−11). The first measurements of a membrane potential in living cells were performed in the ciliate *Paramecium*.[12] The bacterial potassium channel KcsA was the first ion-selective channel to be characterized by X-ray crystallography,[13] and it is now understood that bacteria have a wide array of ion-specific, mechanosensitive, and water channels.[14] Investigations into plant and microbial ion channels not only inform our understanding of basic cellular physiology but also may be instrumental in engineering defenses against microbial pathogens and in crop improvement.[15−17]

Ion channels can be classified according to homology-based family groupings or functional characteristics such as ion selectivity or gating stimulus (in addition to other more subtle behaviors such as conductance, adaptation, and opening or closing kinetics). Many channels are specific to the ion or small molecule that they allow to pass (KcsA has a 1000-fold preference for K^+ over Na^+ ions[18]), while others are not [the bacterial mechanosensitive ion channel of large conductance (MscL) has no ionic preference at all[19]]. Channel conductance, the ease with which current passes from once face of the channel pore to the other, can range over several orders of magnitude in different channel types and organisms. For example, the aforementioned MscL has one of the largest conductances measured, up to 3 nS,[19] while the small potassium (SK) channels associated with Parkinson's disease have a conductance of only 10 pS.[20] The burst of ion flux that results from the rapid opening of an ion channel (occurring on the order of milliseconds) can have several downstream effects: a change in membrane potential, which can serve as a signal itself by exciting other channels; a burst of intracellular Ca^{2+}; or the normalization of ion concentrations across a membrane to control cell volume. Many ion channels open (or "gate") under only certain conditions, such as altered transmembrane voltage, binding of a small ligand, or mechanical force. Mechanosensitive (MS) channels, whose principal gating stimulus is mechanical force, are considered here.

Mechanosensitive Ion Channels. *Gating Models.* How force administered to a cell is delivered to a mechanosensitive channel and how the channel subsequently converts that force into ion flux are important questions requiring the purposeful integration of genetic, biochemical, structural, and biophysical

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approaches. Three simplified models have been proposed for the gating of channels that act directly as mechanoreceptors (that is, there is not an intermediary between the force perception and the channel).[21−23] These models are described below and illustrated in Figure 1.

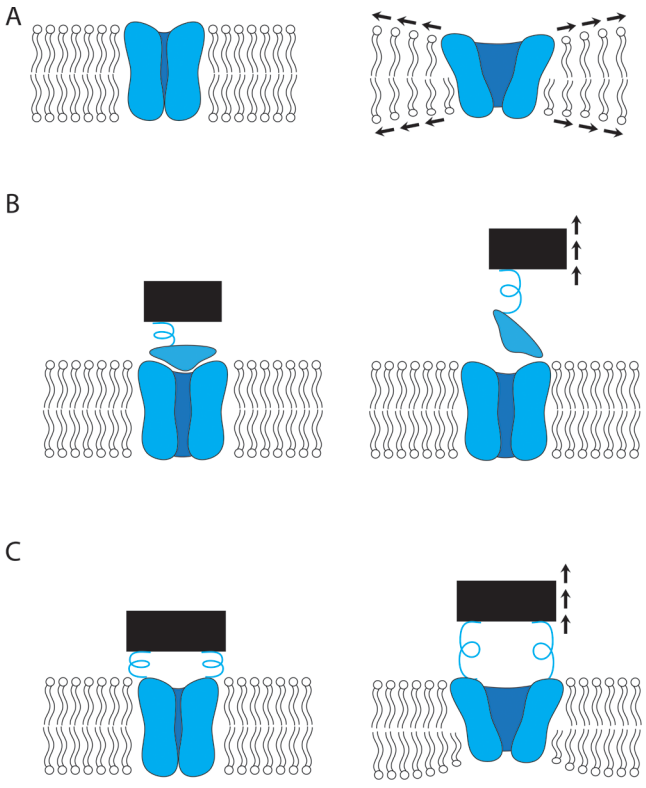

Figure 1. Schematic representation of models for mechanosensitive channel gating. (A) Intrinsic bilayer model, in which lateral membrane tension favors the open state of the channel. (B) Tethered trapdoor model, in which a tether to an extracellular (in this case) component exerts force on the channel, leading to its gating. (C) Unified model, in which a tether to an extracellular component leads to reorientation of the channel within the membrane bilayer, thereby gating it.

Intrinsic. In the intrinsic bilayer model (Figure 1A), force is conveyed to the channel directly through the planar membrane in which it is embedded. Biophysical modeling approaches have identified a number of factors that may favor the closed state of the channel under low membrane tensions, including the energetic cost associated with hydration of the channel pore,[24] and the cost of membrane deformation at the perimeter of the channel.[25] A channel can deform the surrounding membrane because of mismatch between the thickness of the membrane and the thickness of the hydrophobic domain of the channel. In addition, the membrane (which has a compressibility modulus lower than that of the channel[26]) can be locally distorted or bent as it conforms to the shape, or profile, of the embedded channel.[22,27,28] The energy cost associated with these membrane deformations increases upon channel opening, as the cross-sectional area, and therefore the perimeter, of the channel expands. However, loading the membrane with tension through a patch pipet or osmotic pressure can offset this energy cost; under these conditions, the open state is favored. Importantly, membranes are active participants in the gating of MS channels, and the pressure exerted by the lipid on the channel is a critical component of the intrinsic bilayer model.[29] This model is supported by experimental evidence showing that the fluidity, thickness, and curvature of the membrane influence the gating characteristics of MS channels.[30−32]

Tethered. There has long been speculation that mechanotransduction by hair cells of the vertebrate inner ear is mediated by the action of tethers comprised of cadherin and protocadherin (called "tip links") on transducer channels located in the hair cell plasma membrane (reviewed in ref 33). In the tethered trapdoor model (Figure 1B), force is conveyed to the channel through tension applied to other cellular components, such as the actin or microtubule cytoskeleton and/or the extracellular matrix. Displacement of the cellular component pulls on the channel through the tether, thereby triggering its opening. The unified model proposes that rather than opening a trapdoor, pulling on the tether leads to reorientation of the channel within the lipid bilayer, which results in channel gating in response to the membrane deformation and tension forces described above for the intrinsic model (Figure 1C).[23,34,35] Finally, the hybrid model suggests that force-gated channels could be embedded in a cholesterol-rich platform that is in turn tethered to the cytoskeleton.[36] In the latter two models, as with the intrinsic bilayer model, the biophysical properties of the membrane make important contributions to the lowest-energy conformation of a MS channel and can either restrict or facilitate changes in state.

Electrophysiology and Model Systems. The first observations of ion flux in response to mechanical stimuli quickly followed the development of the patch-clamp technique in the early 1980s. This technique allows one to record the current passing across a small patch of membrane tightly sealed to the tip of a thin glass capillary pipet (reviewed in ref 37). A key aspect of this technique is the formation of a high-resistance "gigaseal" between the membrane and the glass (on the order of ≥1 GΩ). When positive or negative pressure is applied to the membrane patch through this glass recording pipet, the membrane (and any associated cytoskeletal components) is deformed. The opening and closing of individual mechanically gated ion channels can then be observed over time.[38,39] Early patch-clamping experiments resulted in the identification of stretch-activated ion channels in animal cells known to be specialized for mechanical perception.[40−43] Similar activities were soon identified in nonspecialized cells,[41,44] leading to the proposal that sensitivity to mechanical stimuli might be a basic cellular feature.[26,45] In the decades since these first studies, many families of MS channels have been identified and characterized in bacteria, plants, animals, and archaea (reviewed in refs 46−48). MS channels can be activated by membrane tension introduced through the patch pipet as described above, by the swelling associated with hypoosmotic shock, or by treatment of cells with membrane-bending amphipaths. Their function has been investigated in endogenous membranes and in a variety of heterologous systems and even reconstituted into artificial membranes. Leading the way in many of these studies is a suite of bacterial channels that are arguably the best studied and best understood mechanoperceptive proteins at the functional, structural, and biophysical levels.

***Escherichia coli* MscL, MscS, and MscM.** *Identification.* Identifying MS channels in bacteria by electrophysiological analysis at first presented several challenges as an *E. coli* cell is smaller than the diameter of a typical patch pipet tip and has a peptidoglycan layer between the inner and outer mem-

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branes.[49,50] This problem was solved by treating cultures with an inhibitor of cell division and then enzymatically digesting the peptidoglycan layer. These treatments result in the production of "giant *E. coli* protoplasts" amenable to patch-clamp electrophysiology.[51] Using this approach, the Kung group measured current induced in response to membrane stretch in *E. coli* and observed a robust tension-sensitive channel activity.[49] Subsequent studies established that at least three distinct channel activities can be detected in the inner membrane of *E. coli*: the mechanosensitive channels of large, small, and mini conductances (MscL, MscS, and MscM, respectively). MscL, MscS, and MscM activities each have different conductances (3, 1, and 0.3 nS, respectively) and are activated at decreasing thresholds of pressure.[19,52−54]

Cloning. It is now established that the three classic activities of the *E. coli* membrane, MscL, MscS, and MscM, represent a complex combination of activities provided by two distinct families of MS channels. The *E. coli mscL* gene was cloned through a fractionation−reconstitution and microsequencing strategy[55] and found to be essential and sufficient for MscL activity. The *mscS*/*yggB* gene was identified through a combination of forward and reverse genetic approaches, and along with *mscL*, it underlies the primary response of an *E. coli* cell to rapid increases in membrane tension.[56] While the MscS and MscL proteins are structurally and evolutionarily unrelated, at least part of the originally observed MscS activity can now be attributed to the action of another channel that is homologous to MscS, now termed kefA/MscK[57] (for more on MscK, see below). When MscL[55] or MscS[58] channels are reconstituted into artificial liposomes, both show characteristics indistinguishable from those of native *E. coli* membranes, indicating that neither requires additional cellular structures for mechanosensitivity. Thus, both MscS and MscL are gated in direct response to lipid bilayer deformation, as in the intrinsic bilayer model (Figure 1A). Relatively less is known about MscM, though recent reports have demonstrated that YjeP and YbdG, two more homologues of MscS, are likely to underlie this elusive activity.[59,60]

Physiological Function. Bacterial cells are found in a variety of dynamic environments, frequently requiring them to adapt to changing osmotic conditions. To maintain turgor pressure during exposure to hyperosmotic stress, bacterial cells accumulate osmolytes that are compatible with cellular metabolism.[61] On the other hand, a sudden shift to hypoosmotic conditions will cause a rapid influx of water across the lipid bilayer, leading to increased membrane tension (reviewed in refs 39 and 62). It has been estimated that a mere 20 mM decrease in external osmolarity can result in membrane tensions that approach lytic levels if unrelieved.[39] A hypoosmotic shock of this type might occur when soil bacteria are caught in the rain, when marine bacteria migrate to freshwater, or when enteric bacteria are transmitted through excrement. Without a rapid response, these shocks would lead to a compromised cell wall, leaving the cell vulnerable to lysis.[63]

It had long been proposed that bacterial cells were capable of relieving this type of environmental hypoosmotic stress by facilitating the exit of osmolytes from the cell, thus ensuring the physical integrity of the cell under increased turgor.[50,61,64] We now know that the primary mechanism for hypoosmotic shock survival is the activation of MS channels, which allows the diffusion of nonspecific osmolytes out of the cell, relieving membrane tension and preventing cellular lysis. *E. coli* strains with lesions in both *mscL* and *mscS* show reduced levels of survival of hypoosmotic shock, though single mutations have no discernible effect.[55,56] Mutants lacking YbdG also show a small defect in osmotic shock survival,[59] and the overexpression of YjeP promotes survival in the absence of all other MS channels.[60] Thus, these bacterial MS channels are often termed osmotic "safety valves"[65] and have been proposed to provide a graded series of responses, allowing the bacteria to tune their response to different osmotic challenges under different environmental or developmental conditions.[14,50,53,57,66]

■ CONSERVATION AND DIVERSITY OF MSCS AND MSCS-LIKE CHANNELS

The classic mechanosensitive channels from *E. coli* described above serve important biological functions, and MscL and MscS have also become leading model systems for the study of MS channel structure and function. Here we focus on the structure and function of the bacterial mechanosensitive channel MscS and its homologues in *E. coli*, in other microbes, and in eukaryotes. Several excellent reviews on MscL have recently been published.[62,67,68]

Structure. Crystallographic studies of MscS structure are beginning to answer the fundamental question of how mechanosensitivity is achieved in MscS-type channels (recently reviewed in ref 69). At present, five structures of prokaryotic MscS homologues have been determined: wild-type *E. coli* MscS (*Ec*MscS) in both open and nonconducting [not necessarily closed (see below)] conformations,[70−72] a point mutation of *Ec*MscS that likely represents the open conformation, and MscS homologues from *Thermoanaerobacter tengcongensis* (*Tt*MscS)[73] and *Helicobacter pylori* (*Hp*MscS)[70] in nonconducting conformations. Four of these structures are shown in Figure 2. A cartoon representation of each is shown from the side (left panel), and both cartoon and space-filling models are shown from the periplasmic surface (middle and right panels, respectively). A fragment containing the three TM domains and the upper vestibule from a single monomer of each of these structures (including amino acids 27−175 for MscS) is shown in Figure 3. Despite the inevitable possibility of artifacts associated with packing contacts and protein−detergent interactions,[23,74,75] these structures provide an invaluable source of information about the molecular mechanism of gating and the relationship between channel structure and electrophysiological behavior.

Nonconducting and Open Conformations of EcMscS and Its Homologues. Nonconducting Conformations. The first crystal structure of *Ec*MscS was determined by the Rees group at 3.7 Å resolution[71,72] (Figure 2A) and revealed a homoheptameric channel with three transmembrane α-helices per monomer and a large, soluble C-terminal domain. This oligomeric state and topology were subsequently verified experimentally.[76−78] As shown in Figure 3, each monomer contributes three N-terminal transmembrane (TM) α-helices to the transmembrane region. TM1 (residues 28−60) and TM2 (residues 63−90) face the membrane, while TM3 (residues 93−128) lines the channel pore (the residues assigned to each helix are as in ref 69). One striking feature of the structure is a sharp kink at Q112/G113, which divides TM3 into TM3a, which is roughly perpendicular to the membrane, and TM3b, which is almost parallel to the membrane (Figure 3A). The narrowest constriction of the pore has a diameter of 4.8 Å and is created by two rings of leucine residues (L105 and L109) with inward-facing side chains. These hydrophobic rings are proposed to prevent the wetting of the pore and thereby serve

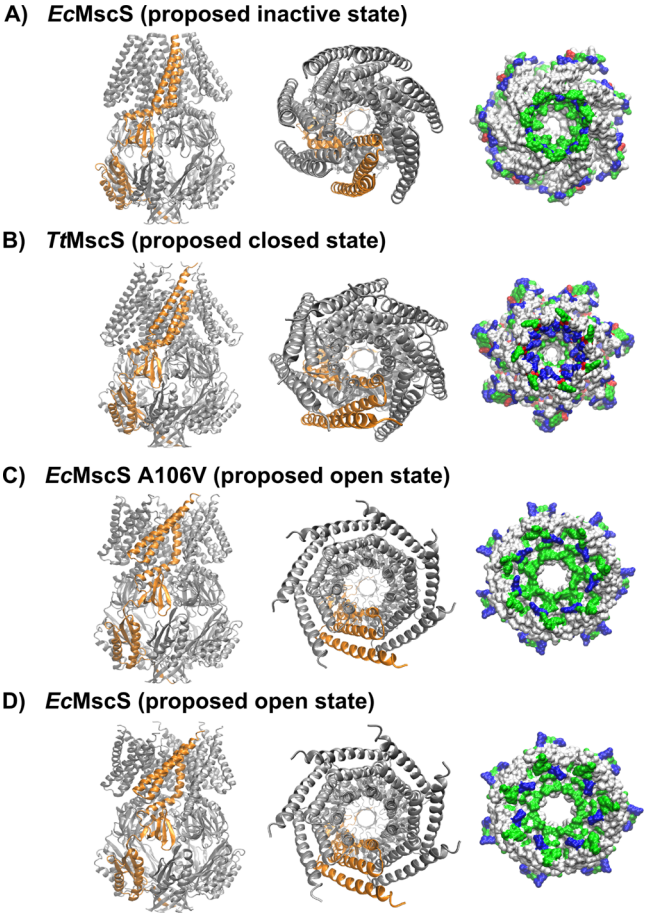

Figure 2. Crystal structures of *E. coli* MscS and homologues. (A) *Ec*MscS in an inactive, nonconductive state (PDB entry 2OAU[72]). (B) *Tt*MscS from *T. tengcongensis* in a closed state (PDB entry 3UDC[73]). (C) A106V *Ec*MscS mutant in an open state (PDB entry 2VV5[84]). (D) *Ec*MscS in an open state (PDB entry 4HWA[70]). The left panel shows side views of the heptameric channel. The middle panel shows views from the periplasmic side. The right panel shows space-filling representations of the pore from the periplasmic side; the channels are truncated at I175 (for *Ec*MscS) for an unobstructed view. Basic residues are colored blue, acidic residues red, polar residues green, and nonpolar residues white. The images were generated with VMD (University of Illinois, Urbana, IL).

as a "vapor lock" to the movement of ions through the channel.[79,80] Mutational analysis of L105 confirmed its importance in maintaining the closed state.[76] The C-terminal region of each monomer contributes to a large hollow structure here termed the "vestibule". The vestibule comprises seven side portals and one axial portal located at the base of the vestibule, formed by a seven-stranded β-barrel.

Originally thought to be the open conformation, this structure it is now generally agreed to represent a nonconducting state. It is unlikely to represent the normal closed conformation, because TM1 and TM2 are not in contact with TM3, an expected requirement for tension-sensitive gating (see the section on force sensing below).[39,81] A number of molecular dynamics (MD) simulations further support this conclusion.[79,82,83] The recently reported structures of *Tt*MscS (Figure 2B) and *Hp*MscS (not shown) exhibit transmembrane helix organizations and pore sizes similar to those of the original *Ec*MscS structure and therefore are also considered to represent nonconducting states.[70,73] The C-terminal vestibule of *Tt*MscS has several differences in structure versus that of *Ec*MscS, which are shown to modulate the conducting properties of the channel and are discussed below.

Open Conformations. Though invaluable for establishing the basic structure of MscS, nonconducting structures give limited insight into the channel's gating mechanism. In a directed attempt to determine the structure of MscS in an alternate conformation, the Booth and Naismith groups crystallized the A106V point mutation of *Ec*MscS at 3.45 Å resolution[84] (Figure 2C). The resulting structure has a substantially increased pore size (approximately 13 Å in diameter) because of a rearrangement of transmembrane helices. TM1 and TM2 are angled away from TM3b and the channel core, while TM3a is tilted away from the plane of the membrane and rotated slightly away from the pore (compare panels A and C of Figure 3). TM3b and the upper vestibule are mostly unchanged compared to the nonconducting structures. These rearrangements place the vapor lock residues out of the pore, as previously predicted on the basis of experimental and modeling data.[85−87] A pulsed electron−electron double resonance (PELDOR) approach[88] revealed that two *Ec*MscS mutants, spin-labeled at D67C (PDB entry 4AGE) or L124C (PDB entry 4AGF), took a similar conformation in solution, indicating that it is not an artifact of crystal packing or of the particular A10V mutation.[89] Further confirmation that the A106V structure properly resembles the open state comes from a recent report describing wild-type *Ec*MscS solubilized in a different detergent (β-dodecyl maltoside instead of fos-choline-14), at a resolution of 4.4 Å[70] (Figure 3D). This structure closely resembles the A106V *Ec*MscS structure, establishing a solid consensus regarding the open state structure of *Ec*MscS.

Gating Mechanism. Despite having multiple crystal structures attributed to different states of MscS, as well as an array of mutational and functional data that have identified functionally important residues, the actual mechanism of transition between closed and open states is still not completely clear. While several models have been proposed on the basis of MD simulations[86,90] and electron paramagnetic resonance (EPR) spin labeling,[87] the model that is currently favored is one in which membrane tension induces the rotation and tilting of TM1 and TM2 as a whole, immersing them more deeply into the surrounding lipid bilayer. This movement pulls TM3a away from the pore until it is oriented almost normal to the membrane plane, effectively removing the L105 and L109 vapor lock side chains and opening the channel to ion flux.[69,84] In all of the crystal structures described above, the positioning of TM1 and TM2 with respect to each other is the same, as if they act like a rigid lever (compare panels A and B of Figure 3 to panels C and D). Assuming that the newly obtained crystal structures described above indeed represent nonconducting and open states, the "rigid-body" movement model of transition into the open state may be considered the most probable.

Lipid−protein interactions must occur at the periphery of the channel, which in MscS is likely to be comprised of TM1 and TM2. Hydrophobic residues in the protein−lipid interface of TM1 and TM2 were shown in several site-directed mutagenesis studies to affect tension sensitivity and osmotic shock protection.[91,92] In addition, an interaction between F68 in TM2 and L111 in TM3 was shown by electrophysiology and mutational analysis to be of critical importance for the transmission of force from lipid-facing helices to the pore region; disruption of this interhelical contact results in channel inactivation.[81] These data are consistent with a model in which

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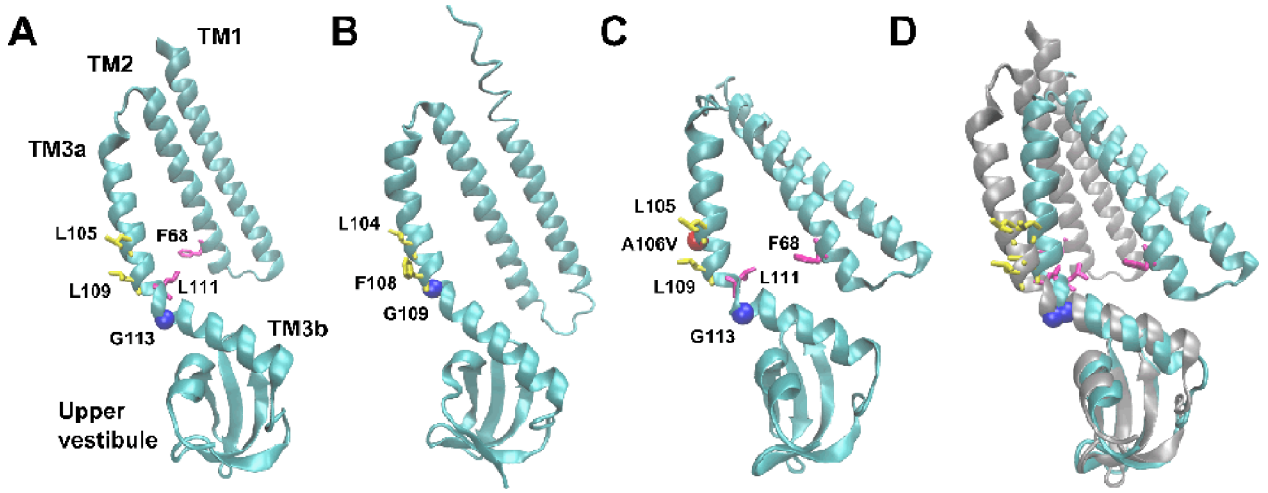

Figure 3. Conserved region of *Ec*MscS and *Tt*MscS monomers in different conformations. A single monomer of (A) *Ec*MscS (amino acids 27−175) in a nonconducting state (PDB entry 2OAU[72]), (B) *Tt*MscS (amino acids 13−175) in a nonconducting state (PDB entry 3UDC[73]), and (C) *Ec*MscS A106V (amino acids 25−175) in an open state (PDB entry 2VV5[84]). (D) Superposition of panel A with a single monomer of *Ec*MscS (amino acids 27−175) in an open state (PDB entry 4HWA[70]). The kink-forming residues G113 (*Ec*MscS) and G109 (*Tt*MscS) are represented as blue spheres, and the A106V mutation is shown as a red sphere. The vapor lock residues L105 and L109 are colored yellow. F68 and L111, residues proposed to mediate the TM2−TM3 interaction,[81] are colored magenta. Images were generated with VMD (University of Illinois).

TM1 and TM2 serve as a tension sensor, transmitting force from the membrane to TM3; subsequent rearrangement of TM3 helices results in channel gating. It is intriguing to consider MscS homologues that possess additional N-terminal transmembrane helices (for several examples, see Figure 4). Additional helices may shield TM2 and TM3 from the lipid environment of the membrane or serve as tension sensors themselves, transmitting force to the pore-lining helix through a different (yet unknown) mechanism.[93]

Contributions by the C-Terminus. Though the structure of the C-terminal vestibule is virtually unchanged in all the crystal structures assigned to open and nonconducting states of *Ec*MscS, other evidence indicates that this portion of the channel may be subject to conformational changes during opening, closing, inactivation, and desensitization transitions. Analyses of multiple deletion and substitution mutants have established that the vestibule is important for channel function and stability,[76,94,95] and that interactions between the upper surface of the vestibule and the TM domain can affect gating as well as inactivation behavior.[96−99] Cosolvents that induce compaction of the C-terminal domain have been shown to facilitate MscS desensitization and inactivation,[100] while experiments utilizing fluorescence resonance energy transfer to quantify the diameter of the cytoplasmic domain showed that it swells during gating.[101] Taken together, these data indicate that gross structural remodeling of the vestibule and its interactions with the transmembrane domain likely accompany inactivation and gating cycles, and there has been speculation that the C-terminus may serve as a sensor for molecular crowding in the cytoplasm.[102]

In addition, the C-terminal vestibule appears to serve as an ion selectivity filter. While MscL forms a large, completely nonselective pore, MscS is slightly anion-selective, preferring Cl^- ions to K^+ ions by a factor of as much as 3 (P_{Cl^-}:P_{K^+} = 1.2−3).[58,103−105] Ions likely do not enter the vestibule through the axial β-barrel, as the portal that it forms is too narrow (1.75 Å in its narrowest part); rather, they probably travel through the seven side portals into the vestibule and then cross the pore. MD simulations suggest the vestibule serves to filter and balance charged osmolytes prior to their release from the cell,[106] and it was recently demonstrated that an electronegative domain adjacent to the side portals contributes to anion selectivity, likely by hindering the passage of cations.[107] Another correlation between the structure of the C-terminus and ion selectivity comes from the functional study of *Tt*MscS.[73] Compared to *Ec*MscS, *Tt*MscS has smaller side portals but a much wider axial portal; at the same time, it has a much higher selectivity for anions. A version of *Tt*MscS in which the axial β-barrel sequence (amino acids 271−282) was replaced with the corresponding portion of *Ec*MscS lost this preference for anions. Taken together, these data indicate that both the β-barrel and the C-terminal vestibule are important determinants of channel behavior.

Thus, the five independently derived crystal structures of bacterial MscS homologues available to date have revolutionized our understanding of the overall architecture of bacterial MscS homologues, provided context for the interpretation of mutagenic data and MD simulations, and established a sophisticated foundation for improving our understanding of the gating cycle. We note that no crystal structures have yet been reported for archaeal or eukaryotic MscS homologues; such a structure would be a major step forward for those interested in the evolutionary diversification of this family of proteins.

Evolutionary History. The MscS protein superfamily is vast and diverse, with members found in most bacterial, protozoan, archaeal, some fungal, and all plant genomes so far analyzed.[108−114] However, MscS family members have not yet been found in animals. It has been suggested that MS channels first evolved in an ancestor common to all cell-walled organisms and have been maintained throughout these lineages as a solution to osmotic stress and regulation of turgor pressure.[108,109,115] Another explanation is that the membrane reservoirs of animal cells allow hypoosmotic swelling without producing membrane tension or that mammalian membranes do not stretch because of their close association with the cytoskeleton.[116,117] Alternatively, MscS homologues could

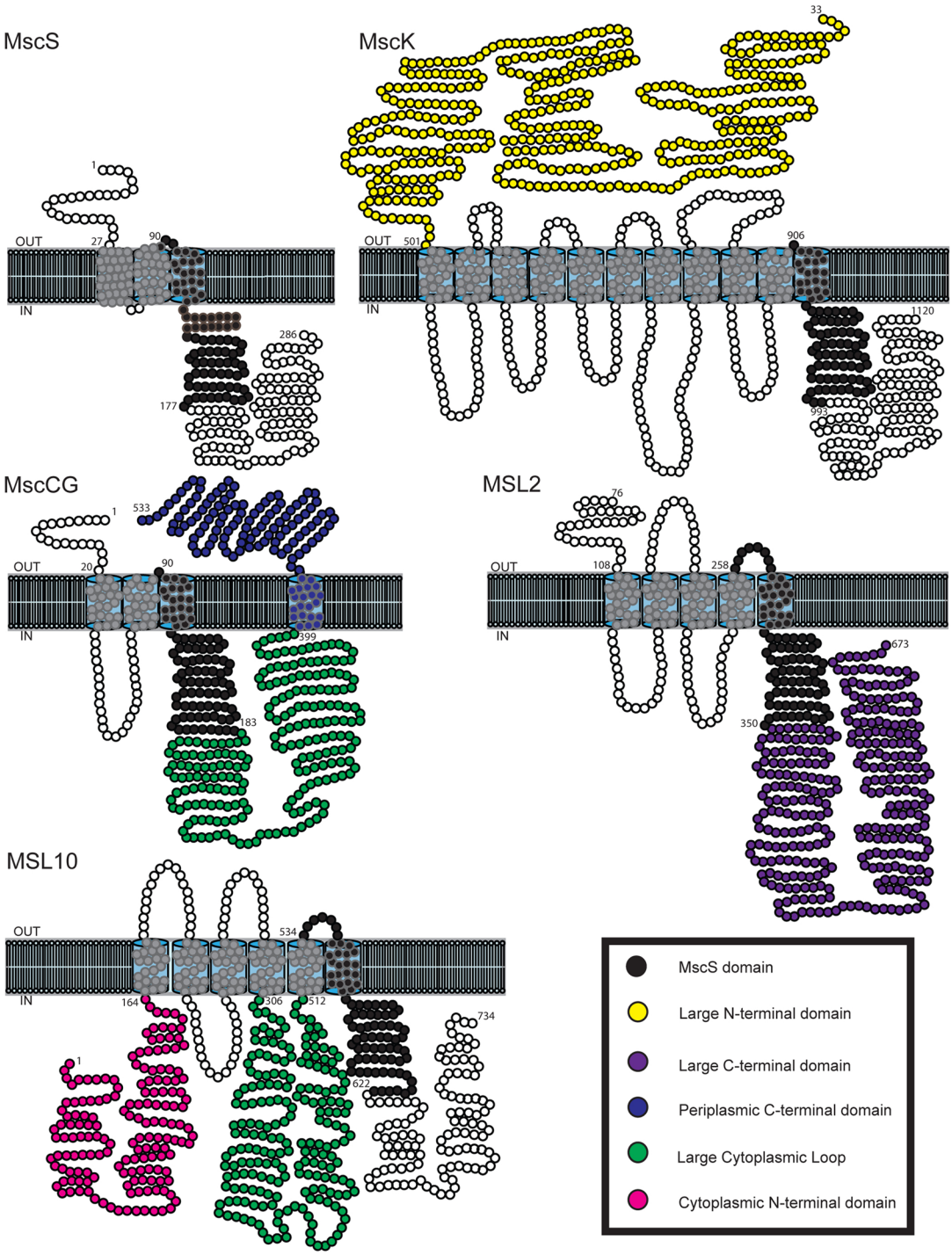

Figure 4. Monomer topologies of representative MscS family members. MscS monomer topology was rendered on the basis of the work of Naismith and Booth.[69] The domain conserved among all MscS homologues is colored black; other domains are colored as indicated in the legend. For the sake of clarity, TM3b of MscS is represented outside the lipid bilayer. MscK and MscCG topologies were predicted with TOPCONS (http://topcons.net/) and ARAMEMNON (http://aramemnon.botanik.uni-koeln.de/) used for MSL2 and MSL10. Processed versions of MscK and MSL2 are presented.

simply be unrecognizable in animal genomes by current homology-based searches.

Mapped onto the MscS structure, the conserved domain comprises the pore-lining helix (in MscS, this is TM3) and the upper part of the cytoplasmic vestibule. Outside of this domain, MscS family members vary greatly in sequence and topology. The number of predicted TM helices for MscS family members ranges from 3 to 12, and a variety of conserved domains, including those associated with the binding of Ca^{2+} and cyclic nucleotides, have been identified in some subfamilies.[56,108,118,119] Furthermore, multiple MscS homologues are frequently identified within a single organism (including many bacterial and all plant genomes analyzed to date), suggesting that functional specialization of MscS homologues has evolved both between species and within a single organism. Our current understanding of the physiological function of MscS homo-

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Table 1. Physiological Functions of MscS Family Members

	organism	gene name	no. of amino acids	physiological function	mutant phenotype	subcellular localization	refs
Prokaryotes	*Escherichia coli*	*YggB* (*MscS*)	286	release of ions during hypoosmotic shock	*mscS mscL* mutant exhibits loss of viability during osmotic downshock; *mscS mscK* mutant has complete loss of MscS channel activity	plasma membrane	56
		MscK (*KefA*)	1120[a]	release of ions in high-K^+ environments	*mscS mscK* mutant has complete loss of MscS channel activity	plasma membrane	56, 125
		YbiO	741	release of osmolytes in high-NaCl environments	*ybiO* mutant has loss of 20 pA channel activity	plasma membrane	60
		YjeP	1107	release of ions during hypoosmotic shock	*yjeP* mutant has loss of 7.5−13 pA channel activity	plasma membrane	60
		YnaI	343	NR[b]	*ynaI* mutant has loss of 2 pA channel activity	plasma membrane	60
	Campylobacter jejuni	*Cjj0263*	627	osmotic protection and host colonization	*cjj0263* has decreased viability after osmotic downshock; *cjj0263 cjj1025* mutant exhibits impaired chick ceca colonization	plasma membrane	136
		Cjj1025	523	host colonization	*cjj0263 cjj1025* mutant exhibits impaired chick ceca colonization	plasma membrane	136
	Bacillus subtilis	*YkuT*	267	osmotic protection	*mscL ykuT* mutant strain has increased sensitivity to osmotic downshock	plasma membrane	127, 128
	Corynebacterium glutanicum	*MscCG*	533	involved in betaine and glutamate efflux	*mscCG* mutant is impaired in betaine efflux during hyper- and hypoosmotic shock and exhibits a 70% decrease in the extent of glutamate export	plasma membrane	130−133
	Synechocystis sp. PCC 6803	*PamA*	680	involved in the transcriptional control of sugar and nitrogen metabolism genes	*pamA* mutant is glucose-sensitive and shows decreased levels of nitrogen response genes and stress sigma factor SigE	NR[b]	138
Eukaryotes	*Arabidopsis thaliana*	*MSL2*	673[a]	plastid osmotic stress response; division ring placement	*msl2* null mutants show defective leaf shape; *msl2 msl3* mutant has enlarged, round, nongreen plastids and enlarged chloroplast exhibiting multiple division rings	plastid envelope	124, 142, 143, 155
		MSL3	678[a]	plastid osmotic stress response; division ring placement	*msl2 msl3* mutant has enlarged, round, nongreen plastids and enlarged chloroplast exhibiting multiple division rings	plastid envelope	124, 142, 143
		MSL4	881	NR[b]	loss of predominant MS channel activity in the root of the *msl4 msl5 msl6 msl9 msl10* quintuple mutant	NR[b]	158
		MSL5	881	NR[b]	refer to *MSL4*	NR[b]	158
		MSL6	856	NR[b]	refer to *MSL4*	NR[b]	158
		MSL9	742	NR[b]	*msl9* null mutant is associated with a loss of 45 pS activity in root protoplast	plasma membrane	158
		MSL10	734	NR[b]	*msl10* null mutant is associated with a loss of 137 pS activity in root protoplast	plasma membrane	158
	Chamydomonas reinhardtii	*MSC1*	522	chloroplast organization	RNAi-mediated knockdown lines show reduced chlorophyll autofluorescence and loss of chloroplast integrity	chloroplast envelope	141
	Schizosaccharomyces pombe	*Mys1*	1011	involved in regulating intracellular Ca^{2+} and cell volume during hypoosmotic stress	*mys1*$^-$ *mys2*$^-$ mutants show decreased viability during osmotic downshock and treatment with $CaCl_2$	perinucler ER	135
		Mys2	840	involved in regulating intracellular Ca^{2+} and cell volume during hypoosmotic stress	*mys2*- and *mys1*- *mys2*-mutants show decreased viability during osmotic downshock and treatment with $CaCl_2$	cortical ER	135

[a]Unprocessed protein. [b]Not Reported.

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logues from bacteria, fungi, plant cells, and plant organelles is described below and summarized in Table 1.

Physiological Function. While it has been clearly established that MscL and MscS serve to protect cells from extreme environmental hypoosmotic shock, it is becoming evident that the functions of the members of this family may be more complex. An emerging theme is that MscS homologues have evolved specific functions tailored to the needs of the organism, including the release of specific cellular osmolytes in response to specific environmental or developmental osmotic triggers.

Prokaryotes. E. coli. We know by far the most about the six MscS family members encoded in the *E. coli* genome (MscS, MscK, YjeP, YbdG, YbiO, and YnaI).[56] Research into their physiological roles suggests that they all serve to release osmolytes from the cell under hypoosmotic stress but that their function is only required for cell viability under specific conditions. Even MscS may serve specialized roles, as MscS protein levels fluctuate. MscS levels are elevated during growth at high osmolarity, possibly a preemptive method of dealing with an impending downshock, and during the stationary phase, perhaps to deal with the osmotically vulnerable state of cell wall remodeling.[120,121] MscS colocalizes with the phospholipid cardiolipin at the poles of the cell,[122] and abnormal division ring placement was observed in an *E. coli* strain lacking MscL, MscS, and MscK,[123,124] suggesting that MscS may also function in bacterial cell division. It was recently reported that the transient increase in the cytosolic Ca^{2+} concentration observed in hypoosmotically shocked bacterial cells is dramatically reduced in an *E. coli* strain lacking MscS, MscK, and MscL, opening up the possibility that MS channels may also impact Ca^{2+} homeostasis.[107]

MscK contributes modestly to cell survival during standard osmotic shock assays,[56,103,125] and its mechanosensitive channel activity requires the presence of K^+ ions in the extracellular solution. It has been proposed that binding of K^+ primes the channel for gating. Such an activity may be required for survival in soils with high concentrations of animal urine or within the kidneys during host infection.[103] The remaining *E. coli* MscS family members (YbdG, YjeP, YbiO, and YnaI) can provide osmotic shock protection when overexpressed in *E. coli*,[59,60] and the latter three activities may simply be expressed at levels too low to contribute under normal laboratory assay conditions. Indeed, the occurrence of the previously uncharacterized mechanosensitive channel activity attributed to YbiO increased dramatically when cells were treated with NaCl prior to being subjected to the patch-clamping technique.[60]

Other Species. The three MscS homologues (*yhdy*, *yfkC*, and *yukT*) of the Gram-positive bacterium *Bacillus subtilis* are dispensable for osmotic shock survival in the laboratory, though the *mscL yukT* double mutant strain exhibits enhanced osmotic sensitivity compared to that of the *mscL* single-deletion strain.[126−128] As *B. subtilis* is found in both the soil and the human gut, there may be specific growth conditions under which these MscS homologues contribute to osmotic homeostasis that are not replicated in the laboratory environment. Other prokaryotic MscS homologues provide tantalizing ideas about the variety of ways in which this family of channels may have evolved to provide osmotic adjustment in response to different environmental and developmental stimuli. The Gram-positive bacterium *Corynebacterium glutamicum* is used in the industrial production of glutamate and other amino acids.[129] Its genome encodes homologues of both MscL and MscS (MscCG/NCgl1221), but neither is required for cell survival in laboratory-based osmotic downshock assays.[130,131] Instead, MscCG is involved in regulating the steady state concentration of glycine betaine (the preferred compatible osmolyte of *C. glutamicum*) in response to both hypo- and hyperosmotic stress.[132] MscCG is also essential for glutamate efflux in response to biotin limitation and penicillin treatment, notably in the absence of hypoosmotic stress.[131,133] Several lines of evidence, including the analysis of loss-of-function and gain-of-function lesions in the predicted pore-lining helix, support a model in which MscCG directly mediates the efflux of glutamate and this efflux is dependent on mechanosensitive channel gating.[131,133−135] Thus, MscCG is likely a mechanically gated MscS homologue that is involved in osmotic adjustment of specific compatible solutes in response to multiple stimuli.

Finally, there are indications that MscS family members are important for pathogenesis and metabolism, perhaps indicating the importance of osmotic adjustment in these processes. Two MscS homologues from the food-borne pathogen *Campylobacter jejuni*, *Cjj0263* and *Cjj1025*, were recently found to be required for colonization of the digestive tract of chicks,[136] and a *Pseudomonas aeruginosa* MscK ortholog has been associated with virulence.[137] PamA, a MscS homologue from the photosynthetic cyanobacterium *Synechocystis* sp. PCC 6803, was reported to interact *in vitro* and *in vivo* with PII, a highly conserved carbon/nitrogen sensor.[138,139] Furthermore, nitrogen response and sugar metabolic genes show altered expression in the absence of *PamA*, suggesting that it may serve to integrate carbon and nitrogen metabolism with osmotic conditions. A recent study of MscL-like and MscS-like activities in *Vibrio cholerae* showed that this species has a high density of MS channels in the cell membrane yet is more sensitive to osmotic shock than wild-type *E. coli*.[140] Taken together, these preliminary studies illustrate how much has yet to be revealed regarding MscS homologue function in the prokaryotic world.

Eukaryotes. While the eukaryotic members of the MscS family have been studied less than their prokaryotic counterparts, recent research offers a few glimpses into their important functions and novel characteristics. Sequence similarities place them into two major classes (described in ref 110). Class II members are predicted to localize to the plasma membrane or intracellular membranes of both plants and fungi. Class I channels, which show a slightly higher degree of sequence conservation with respect to MscS than class II channels, are predicted to localize to endosymbiotic organelles (mitochondria and plastids such as chloroplasts) and are found only in plant genomes.

Class I. Considering the origin of endosymbiotic organelles (the engulfment of a primitive bacterium), the MscS homologues found in their envelopes are likely to have a conserved function as osmotic safety valves, but in this case protecting mitochondria and plastids from fluctuations in intracellular rather than extracellular osmotic concentrations.[16] Mechanosensitive channel (MSC) 1 from *Chlamydomonas reinhardtii* localizes to punctate spots associated with the single plastid found in these cells, and plastid integrity is lost when the *MSC1* gene is silenced by RNAi.[141] To date, MSC1 is the only class I MscS homologue to be successfully characterized by electrophysiology (see below). Like MSC1, MscS-like (MSL) 2 and MSL3 of *Arabidopsis thaliana* localize to distinct foci in the plastid envelope. These two land plant class I homologues are required for normal plastid shape and size and for proper

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Table 2. Single-Channel Properties of MscS Family Members

	species	name	unitary conductance	ion selectivity (P_{Cl}:P_K)	no. of TMHs[a]	identity in the pore-lining domain and upper vestibule to *Ec*MscS (%)[b]	refs
Prokaryotes	*E. coli*	*Ec*MscL[c]	3 nS[d]	nonselective	2	–	55, 157
		*Ec*MscS	1.2 nS[d]/350 pS[e]	1.2–3	3	100	56, 58
		*Ec*MscK	1 nS[d]	<*Ec*MscS	11[f]	32	49, 103
		YjeP	250–400 pS[d]	NR[g]	11[f]	27	60
		YbdG[h]	350–400 pS[d]	NR[g]	5[f]	21	59
		YnaI	~100 pS[d]	NR[g]	5[f]	30	60
		YbiO	~850 pS[d]	NR[g]	12[f]	24	60
	Si. pomeroyi	MscSP	1.04 nS[i]	1.4	3[f]	49	145
	T. tengcongensis	*Tt*MscS	134 pS[d]	8.7	3	29	73
	C. glutamicum	MscCG	328 pS[i]	0.3	4	29	132, 133
	M. jannashii	MscMJLR	2 nS[j]	0.2	5[f]	41	115, 146
		MscMJ	270 pS[j]	0.16	5[f]	36	115
Eukaryotes	*Ch. reinhardtii*	MSC1	390 pS[k]	7	5[f]	32	141
	A. thaliana	MSL10	103 pS[e]	5.9 (P_{Cl}:P_{Na})	6[f]	18	158, 144

[a]Number of transmembrane helices predicted via the TMHMM 2.0 server. [b]Alignments were made using the Kalign algorithm in Unipro UGENE. [c]MscL is not a MscS homologue (added for reference). [d]In 200 mM KCl, 90 mM $MgCl_2$, 10 mM $CaCl_2$, and 5 mM HEPES (pH 7.0). [e]In 96 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES (pH 7.38). [f]Predicted. [g]Not reported. [h]Channel activity was shown for only a V229A mutant of the YbdG-encoded protein. [i]In 250 mM KCl, 90 mM $MgCl_2$, and 5 mM HEPES (pH 7.2). [j]In 200 mM KCl, 5 mM $MgCl_2$, and 5 mM HEPES (pH 7.2). [k]In 200 mM KCl, 40 mM $MgCl_2$, 10 mM $CaCl_2$, 0.1 mM EDTA, and 5 mM HEPES-KOH (pH 7.2).

placement of the plastid division ring.[124,142] The large, round plastid phenotype of the *msl2 msl3* mutant can be suppressed by a variety of genetic and environmental treatments that increase cytoplasmic osmolyte levels, indicating that plastids are under hypoosmotic stress from within the cytoplasm and that MSL2 and MSL3 are required to relieve that stress.[143] Several class I MscS homologues from land plants are predicted to localize to the mitochondria,[110,113] but that study has not yet been reported.

Class II. The identification of MscS homologues in plant genomes[108,109] was exciting for plant biologists because it provided candidate genes for the MS channel activities already known to be widespread in plant membranes.[110] However, while the *Arabidopsis* genome contains seven MSL proteins that are predicated to localize to the plasma membrane and exhibit distinct tissue-specific expression patterns,[108,110] a clear physiological function has yet to be assigned to any [though MSL10 has been characterized by patch–clamp electrophysiology (see below)]. The recent characterization of two endoplasmic reticulum-localized MscS homologues from *Schizosaccharomyces pombe*, Msy1 and Msy2, suggests that these channels may serve as hypoosmotic stress signaling molecules as much as osmotic safety valves.[118] *msy1- msy2-* mutant cells exhibit greater swelling and higher Ca^{2+} influx upon hypoosmotic shock and are more likely to subsequently undergo cell death. Consistent with this idea, we have proposed that MSL10 could play a role in hypoosmotic stress signal transduction through membrane depolarization.[144]

In conclusion, current evidence indicates that members of the MscS superfamily exhibit unique forms of regulation and variations of function. While all are variations on a common theme, action as an osmotic conduit in response to membrane tension, the proteins within this family may have become as diverse as the organisms in which they reside. We anticipate that more precise analyses, under diverse growth conditions and at the single-cell or organellar level, will reveal the role played by these channels in the osmotic homeostasis of cells and organelles.

Electrophysiological Behavior. Besides *Ec*MscS, many MscS superfamily members have been shown to be mechanosensitive, including five others from *E. coli* (MscK, YbdG, YnaI, YjeP, and YbiO)[59,60,103] and three from other bacterial species (*Tt*MscS from *T. tengcongensis*,[73] MscSP from *Silicibacter pomeroyi*,[145] and MscCG from *C. glutamicum*).[132] Two MscS homologues from the archaea *Methanococcus jannaschii*, MscMJ, and MscMJR, have been characterized,[115,146] as have two channels from photosynthetic eukaryotes (MSC1 from *Ch. reinhardtii* and MSL10 from *A. thaliana*).[141,144] Despite striking differences in topology and sometimes very low levels of sequence identity, these channels demonstrate surprisingly conserved behavior in many aspects. Perhaps most striking are their relatively large unitary conductances, 10 orders of magnitude larger than those recorded from most animal ion channels. Their major characteristics are listed in Table 2 and discussed in further detail below. Not included here are MscS-related channels from *B. subtlilis*,[147] *Streptococcus faecalis*,[148] and the bCNG family.[119]

Conductance and Ion Selectivity. The MscS homologues listed in Table 2 all have weak to moderate ionic preferences and single-channel conductances that fall into an approximately 4-fold range. MscSP closely resembles *Ec*MscS in sequence and in channel characteristics,[145] and MscK has a conductance close to that of *Ec*MscS.[56,103] However, some variation is observed among the prokaryotic channels, with a smaller conductance typically associated with more selectivity. MscCG has a single-channel conductance of 0.3 nS, approximately one-third the size of that provided by *Ec*MscS, and prefers cations (P_{Cl^-}:P_{K^+} = 0.3).[132] YjeP has a similar conductance and is also likely to have a preference for cations, as this was the early characterization of MscM activity (P_{Cl^-}:P_{K^+} = 0.4).[53,60] As described above, *Tt*MscS has a single-channel conductance approximately half that of *Ec*MscS and is more strongly anion-selective (P_{Cl^-}:P_{K^+} = 8.7).[73] MscMJ (270 pS) and MscMJLR (2 nS) both exhibit a similar

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preference for cations (P_{Cl^-}:P_{K^+} = 0.16 and 0.2, respectively).[115,146] Eukaryotic channels MSC1 and MSL10 are quite similar to each other: both have conductances that are approximately a third of that of MscS under similar conditions, and both show a preference for anions (P_{Cl^-}:P_{K^+} = 7 and 6, respectively).[141,144,149]

Inactivation and Desensitization. Models of the MscS activation cycle typically include four distinct states: open, closed, inactive, and desensitized.[56,98,100,150] While the latter three states are distinct, both an inactivated and a desensitized channel will manifest themselves as current decay in patch-clamp recordings under a fixed membrane tension. To experimentally distinguish between them, one must either apply an additional pulse of pressure beyond what is required to saturate all the channels in the patch or decrease the tension and apply it once again. In the inactive state, the channel cannot make a transition to the open state under any tension, while a desensitized channel could be gated by the application of an increased tension. While inactivation and/or desensitization under sustained membrane tension has been reported for MscS expressed in several systems,[90,98,149] MscSP, MscCG, MscK, MscMJ, and MscMJLR do not desensitize.[56,103,115,132,145,146] MSL10 does not show any significant signs of inactivation,[144] while MSC1 inactivates at positive membrane potentials, but not at negative.[141] These results leave unclear the physiological relevance of inactivation.[62]

Recent insight into the molecular mechanism and physiological relevance of inactivation came from the discovery that, like channel opening, *Ec*MscS inactivation occurs as a transition from the closed state.[150] The inactivated state is characterized by an ~8.5 nm^2 in-place protein expansion (small compared to the 12−15 nm^2 expansion associated with channel opening). During inactivation, the TM1−TM2 hairpin is thought to bend away from TM3, which stays in the closed arrangement.[72] The result is a channel conformation in which TM1 and TM2 are sterically restricted from transmitting tension to the pore region, and the channel cannot be gated. This behavior may prevent efflux of important solutes from the cell under slightly hypoosmotic conditions. On the other hand, the lack of inactivation from the open state ensures that the population of channels stays open as long as the membrane tension is kept high.[150,151]

Hysteresis. Another feature of mechanosensitive channel behavior is hysteresis, or a difference between the tensions required for opening and closing. *Ec*MscS is routinely observed to close at tensions lower than that at which it opened when tension is rapidly applied and released. However, this behavior disappears when tension is changed more gradually,and is likely the consequence of a relatively slow closing rate (summarized in refs 57 and 152). The eukaryotic channels MSC1 and MSL10 also show hysteresis. These channels close at a tension lower than that at which they opened, and a subpopulation of both types of channels often is observed to stay open even after all membrane tension has been released.[141,144] There are no reports of any functional importance attributed to this phenomenon, but the continuous slow depolarization of the membrane due to channels staying open after membrane tension is relieved could result in the gating of depolarization-activated channels and/or the propagation of a systemic signal.

Thus, despite limited sequence identity, the MscS family members so far characterized share similar basic channel characteristics such as conductance and ion selectivity. Other behaviors observed under patch-clamp processing, such as hysteresis and inactivation and/or desensitization, are more variable and have unclear physiological relevance. One could speculate that the conserved features of these channels reflect their common function (rapid release of osmolytes in response to membrane tension), while their characteristic differences reflect the specific natures of their ecological niches.[60] Additional examples may help to determine the functional range of properties that have been selected by evolution.

Topological Diversity in the MscS Superfamily. The topological complexity of MscS family members (as described above and illustrated in Figure 4) has been taken to imply regulatory complexity,[23,112] and data suggest that this may indeed be the case. Many members of the MscS family contain N- and C-terminal domains dramatically larger than that of MscS, presenting the possibility of additional functions and regulation sites. For example, the unusually large periplasmic N-terminal region of MscK could regulate channel activity by preventing gating in the absence of high K^+ concentrations.[103,153] Removal of the N-terminal region of MscK, including TM helices 1−9, abolishes K^+-dependent gating and promotes its ability to provide protection from hypoosmotic shock.[76] Similarly, the presence of an extra TM helix C-terminal to the pore-forming helix is unique to MscCG and can confer the ability to facilitate glutamate efflux when fused to *Ec*MscS.[133] Proteins comprising the bCNG family all encode a large soluble C-terminal domain containing a cyclic nucleotide-binding domain. This domain has been shown to negatively regulate the mechanosensitive channel activity of one of the family members.[119,154]

The eukaryotic MscS family members show topology that is just as diverse. A variety of physiological functions have been attributed to chloroplast channels MSL2 and MSL3, which contain a C-terminal cytoplasmic domain 3 times the size of the MscS soluble domain.[124,155] Although the regulatory and functional importance of this domain has yet to be confirmed, preliminary evidence suggests that a highly conserved domain within this region is required for proper subcellular localization and channel function *in vivo* (E. S. Haswell, unpublished observations). Class II (plasma membrane- and ER-localized) eukaryotic homologues of MscS, such as MSL10, typically share a common topology of six TM regions, large soluble N- and C-termini, and a large cytoplasmic loop between TM helices 4 and 5,[110,118] suggesting that their conserved structure serves a eukaryote-specific function. The large cytoplasmic regions of many class II proteins suggest a number of possible regulatory mechanisms. For example, Msy1 and Msy2 contain an EF-hand Ca^{2+}-binding motif[156] in the large cytoplasmic loop between TM4 and TM5. Genetic analyses suggest that this region is important for sensing and/or controlling Ca^{2+} influx as well as contributing to channel function in response to hypoosmotic stress.[118]

■ FUTURE DIRECTIONS

As we hope we have demonstrated above, these are exciting times for scientists who study mechanosensitive ion channels. Every new detail regarding the structure, the physiological function, and the biophysical parameters that govern the gating mechanism of *Ec*MscS adds to our understanding of *E. coli* biology and helps elaborate an important model system for the study of mechanosensitivity. Prokaryotic homologues of MscS provide additional examples of the ways in which various bacteria might exploit the membrane tension sensor and osmotic safety valve provided by a MscS family member. The

suggestion that more diverged MscS families may have additional regulatory mechanisms overlaid onto a conserved mechanosensitive channel core is particularly interesting in this regard.[119] The coming years should also bring an improved understanding of the role played by the diverse eukaryotic family of MscS homologues. Eukaryotic cells respond to osmotic stress differently than bacteria, inducing cell signaling pathways in addition to releasing osmolytes.[21] Studies of yeast Msy1 and Msy2 suggest that they might play a role in both of these responses;[118] further investigation will establish this point. New discoveries are also likely as some of the technical challenges associated with the study of mechanosensitive channels are overcome. Approaches to investigating osmoregulation and osmotic stress response in single cells and organelles may reveal phenotypes more subtle than those that can be detected in a bacterial culture or from a whole-plant phenotype. The development of fluorescent biosensors that report on ion flux, pH, transmembrane voltage, and membrane tension could produce unexpected insights into the function of MscS-like mechanosensitive channels in their endogenous cellular context.

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ABBREVIATIONS

MS, mechanosensitive; MscS, mechanosensitive channel of small conductance; MscL, mechanosensitive channel of large conductance; MscM, mechanosensitive channel of mini conductance; MscMJ, mechanosensitive channel of *M. jannaschii*; MscMJLR, mechanosensitive channel of *M. jannaschii* of large conductance and rectifying MSL; MscS-like Msy, MscS from yeast; MscCG, mechanosensitive channel of *C. glutamicum*; MscSP, mechanosensitive channels of *Si. pomeroyi*; TM, transmembrane; P_{Cl^-}, permeability to Cl^- ions; MSC, mechanosensitive channel; EPR, electron paramagnetic resonance; MD, molecular dynamics; PDB, Protein Data Bank.

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